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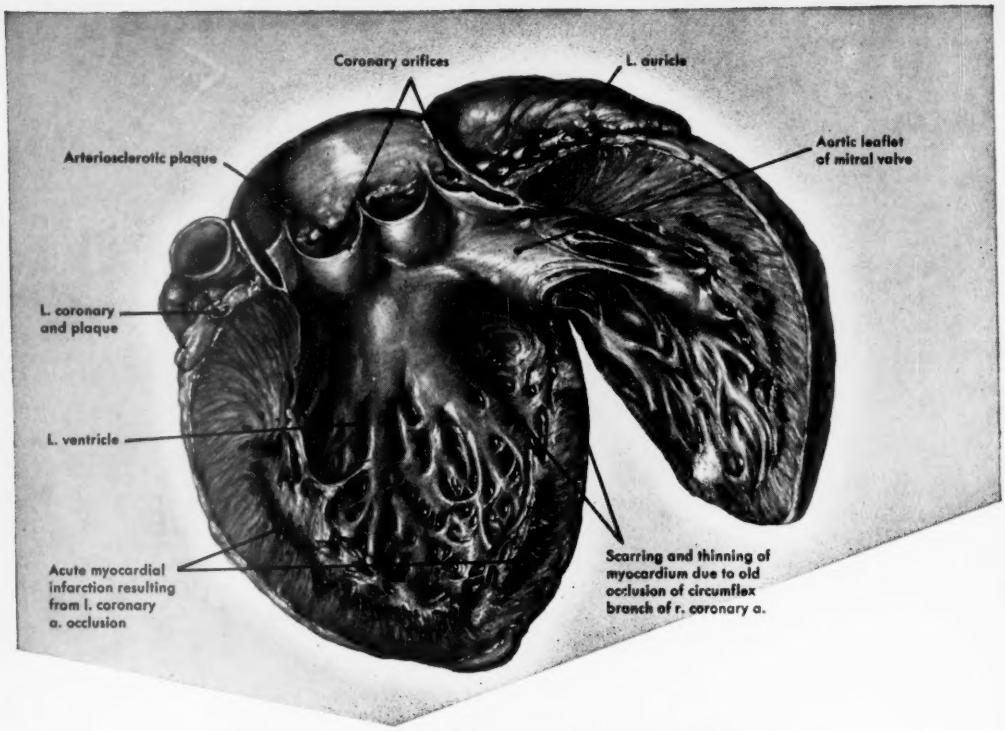
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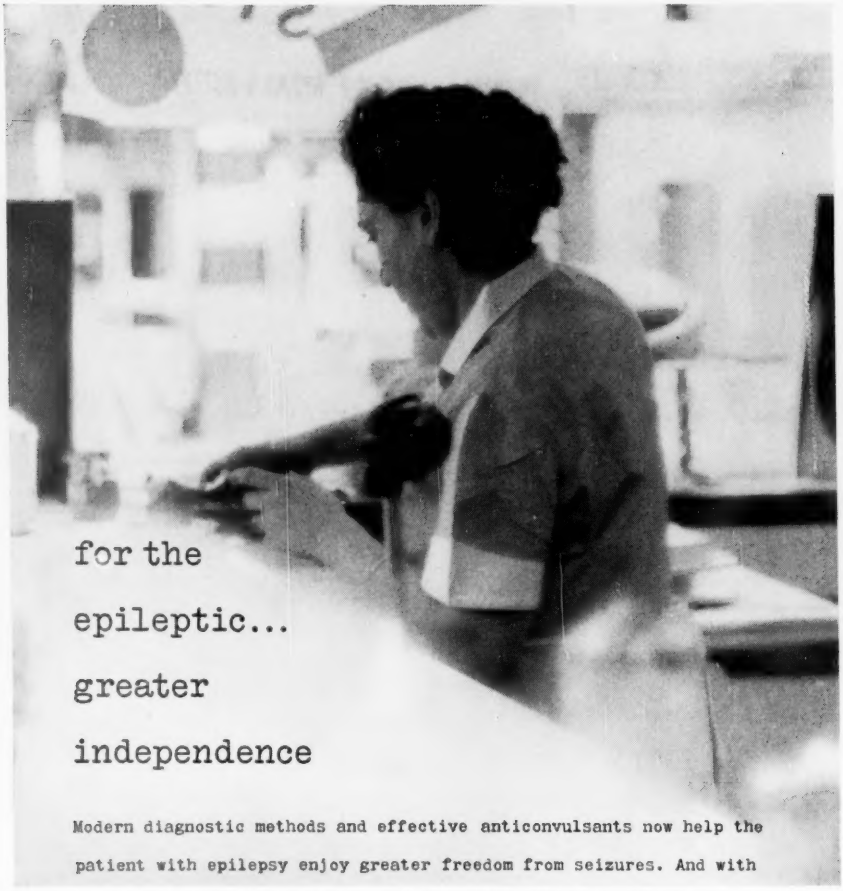

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SOME REMARKS ON THE MANAGEMENT OF DIABETES MELLITUS*

The common practice of speaking of the spontaneous diabetes mellitus of man as a disease is not yet and may never be justified. The term is applied to any condition in which the oxidation of carbohydrate is chronically impaired. The process of "oxidation" is specified in order to exclude disturbances of glycogenesis and glycogenolysis that may accompany disorders of the function of the liver, the thyroid, etc. The term "chronically" is used not in the sense of permanently; disorders with all the characteristics of diabetes mellitus have been known to disappear completely after they have persisted for considerable periods of time. "Chronically" is intended to exclude transient impairments of carbohydrate oxidation, such as those associated with starvation, administration of adrenaline, drugs, etc.

Inherent reactions of intermediary metabolism follow essentially the same procession in all mammals. All utilize similar materials, are controlled by the same enzyme systems, regulated by the same hormones. Nevertheless, species differ sharply from one another in their reactions to diets, to hormones and to disease, injury or removal of organs. The blood sugar of the goat and other ruminants, for example, is low. The dog, inured to a carnivorous diet, is peculiarly tolerant to carbohydrate starvation and resistant to ketosis. The rabbit is unable to utilize protein and fat with facility. After destruction of the islands of Langerhans it fares well, despite glycosuria, if fed carbohydrate; but, if deprived of carbohydrate, rapidly succumbs to ketosis. These distinctions between species are probably referable to differences in the relative speed of reactions of enzymes and of the delivery of or response to hormones. It follows that, although animal experiments may throw great light on the phenomena of intermediary metabolism in health and disease including diabetes, they cannot be translated bodily into terms of human physiology or pathology. To no other field is Pope's line: "The proper study of mankind is man," more appropriate.

The discovery that a disorder closely resembling human diabetes can be produced in man and other animals by removal of the pancreas or destruction of the islands of Langerhans has led many to attribute human diabetes

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to disease of these structures. Positive evidence in support of this view is, however, meager. Human pancreatic diabetes does occur; but lesions of the islands of Langerhans cannot be demonstrated consistently in patients with spontaneous diabetes. When such lesions are found, their severity is not correlated with the gravity of the metabolic disorder. Now that operative removal of the whole pancreas is feasible, it has been possible to study the effects of this procedure in man. As might have been anticipated from studies of other anthropoids and from the intermediary metabolism of humans, after pancreatectomy glycosuria and hyperglycemia can usually be controlled by moderate doses of insulin, 30 to 50 units daily; but, if insulin is withdrawn, severe acidosis rapidly ensues. On the other hand, some patients with clinical diabetes often require far larger doses of insulin to control glycosuria and hyperglycemia, but tolerate withdrawal of insulin without dramatic reactions. While insulin derived from the pancreas has a specific action on the intermediary metabolism of carbohydrate and protects diabetic patients from the evil metabolic consequences of diabetes, it can rectify disorders of these processes that do not originate in the pancreas.

Other endocrine products affect carbohydrate metabolism and have, therefore, been implicated in the etiology of diabetes. While the β-cells of the islands of Langerhans produce insulin which facilitates the utilization of carbohydrate, the α-cells elaborate a product, glucagon, which promotes hyperglycemia and glycosuria. This is not, however, a direct antagonist of insulin and lacks the characteristics required to reproduce the phenomena of diabetes. Its chief action appears to be the promotion of hepatic glycogenolysis. The anterior lobe of the pituitary secretes hormones that diminish the utilization of carbohydrate. Eosinophilic tumors of the gland produce acromegaly. This is usually or often attended by some degree of diabetes, which is indistinguishable, so far as the disorder of carbohydrate metabolism is concerned, from other varieties of clinical diabetes. The "diabetogenic" hormone of the anterior pituitary may exercise a direct anti-insulin effect in the tissues. Some preparations, when given to animals, ultimately produce permanent diabetes associated with destruction of islet tissue. No counterpart of such a condition has been noted in acromegalic patients. The diabetes of acromegaly, like other functional manifestations of the disease, sometimes subsides when the adenoma regresses or degenerates. In any case it is not consistently attended by discernible lesions of the pancreas. Tumors or overactivity of the adrenal cortex and administration of cortisone or adrenocorticotrophic preparations from the anterior pituitary may also provoke in man signs and symptoms of diabetes without demonstrable pancreatic lesions.

Both epinephrine and thyroxine disturb carbohydrate metabolism, but their actions differ from those that arise from absence of insulin. Thyroxine, by accelerating oxidations, imposes upon the organism a greater demand for combustion of carbohydrate. Epinephrine diverts carbohydrate combustion into the anaerobic pathway that leads to formation of lactic acid. Both hyperthyroidism and the discharge of epinephrine aggravate diabetes. While diabetes mellitus cannot be identified consistently with primary disease of any of these endocrine glands, the metabolic disabilities which characterize the disease presumably involve disorders of their activities or the effectiveness of the hormones they secrete.

Diabetes is one of the commonest disorders; its incidence in the population as a whole has been placed as high as 10 per cent. It follows that almost all disorders and diseases are found in company with diabetes, making it difficult to decide whether these coincidences are accidental or significant. There appears to be a definite hereditary tendency to the disorder; but the statistical conclusion that it is a recessive characteristic is open to objection until it has been ascertained that it is a distinct and uniform entity.

Diabetes has been discovered in infants shortly after birth (it has even been claimed that it may occur *in utero*). Its incidence rises gradually in childhood and early adult life; but mounts with increasing acceleration during and after the fifth decade, assuming the shape of the curve usually attributed to senescence. Such a curve prompts the questions: (1) whether this accelerated incidence represents the fruition with time of processes originating earlier in life; (2) whether it indicates the accession of a new disorder or disorders; (3) whether it is merely part of the attrition of the ageing process. The child with diabetes has notoriously run a stormy course, punctuated with acidosis, insulin reactions, and other metabolic vicissitudes. Whether this instability was merely a reflection of the instability of carbohydrate metabolism in childhood or whether the diabetes of childhood was a distinct entity could not be ascertained before the advent of insulin, because up to this time children with diabetes did not grow up. Records relevant to the life history of diabetes began less than 30 years ago. From limited data it appears that the diabetes of childhood tends to become less unstable with advancing age. There is often a renewal of the storm during adolescence; but in the majority of those who survive to full maturity the disorder assumes a more measured course.

As the age curve is followed there is an increasing incidence of vascular disease, with or without hypertension, characterized by obliterating endarteritis of the smaller, and calcification of the larger, arteries, with an especial predilection for the arteries of the cerebrum, the heart, and the lower

extremities, giving rise to vascular accidents in these regions. Furthermore, in a large proportion of diabetics material that appears to be composed of protein is deposited in the glomerular tufts of the kidneys and the eyesight is gradually destroyed by rather distinctive lesions of the retinal vessels. So great is the incidence of vascular disease that it is regarded by many as a consequence of the diabetes. It is, however, a fallacy to presume that because two variables are correlated with one another they have the relations of cause and effect, or without other grounds to assign to one of them the rôle of cause. A plausible argument could be built up to the effect that the vascular disease, not the diabetes, is the primary disorder. The glucose tolerance of patients with vascular disease, including Buerger's disease, is frequently reduced. There is no consistency in the order of appearance of the diabetes and the vascular disease. It is quite possible that they are only different manifestations of a syndrome or diathesis. In fact, this view is rapidly gaining ground at the present time. There is no unassailable evidence that the metabolic disturbance of diabetes conduces to vascular disease (Lukens' single diabetic dog that developed Kimmelstiel-Wilson lesions of the kidneys bears no more weight than the single robin that is no harbinger of spring). Although the incidence of diabetes is correlated with the incidence of vascular disease, the severity of the two conditions is not directly correlated. Indeed, some have contended (as yet without altogether adequate grounds, I believe) that the diabetes of patients with the Kimmelstiel-Wilson complex is relatively benign. The picture is somewhat confused by the fact that the vascular disorders of diabetics do not all follow the characteristic pattern. There is no evidence that diabetes protects persons from the types of vascular disease to which the rest of the population is subject. Indeed, it may render them more vulnerable. Recent reports have indicated that the incidence of pyelonephritis, a major cause of hypertensive vascular disease, in female diabetics far exceeds that of the population at large. I suspect that this may be a product of irritation and infections of the vulva and vagina induced by glycosuria.

Diabetic women seem to be peculiarly prone to toxemias of pregnancy and to bear unusually large children. The etiological rôle of the metabolic disorder in these two phenomena is also open to question; they too may be features of the same or another syndrome or diathesis. Women whose diabetes began after one or more pregnancies have been found to have had a greater than normal incidence of both toxemias and oversize babies in the pregnancies preceding the onset of diabetes.

Neuropathies of a varied nature are unusually frequent in diabetes. These again are unrelated to the severity of the metabolic disorder; their correla-

tion with the vascular disorders is uncertain. The subject is confusion twice-confounded because the known association serves as an evasive mechanism for the medical profession. Pains and disabilities of all kinds are cursorily consigned to the category of neuropathies and diabetics with true neuritides frequently receive less careful scrutiny than would be accorded to other patients with similar disorders.

Depancreatized animals almost regularly develop metabolic cataracts. There was a tendency to attribute these to the absence of the external secretion of the pancreas, since the hypocalcemia of steatorrhea was known to lead to the formation of cataracts. The discovery that cataracts regularly occur in alloxanized animals seems to connect them with the metabolic disability of diabetes. It is now established that the incidence of cataracts in diabetic patients is excessive; but, unlike diabetic animals, the majority of human diabetics survive for years without developing such lesions. On the other hand, a certain number, not necessarily the most severe, develop precocious metabolic cataracts.

These prominent associations have been selected from a great number, verified or alleged, to emphasize the point that human diabetes cannot be treated as a disorder of metabolism alone. Each patient must be scrutinized for these complications if therapy is to be properly directed. Moreover, these associations deserve the most meticulous study, since in them may lie some of the secrets of the nature of human diabetes, whether this be singular or plural. Such a study must be made without inhibitory preconceptions. Statistical analyses of correlations of the phenomena of the disorder are likely to be useful only for purposes of prediction. A careful investigation of the natural history of diabetes promises greater returns. The sad realization has been reached that a large proportion of children afflicted with the disorder follow the pattern of adults with respect to vascular disease. This majority in both groups is, however, no more important than the minority that mysteriously escapes these evils, or, since the life history of the young diabetic is still to be written, has managed to survive without them far beyond the general expectation.

A word on another point to which I shall revert later: it is too early to congratulate ourselves and somewhat ridiculous to blame patients for the incidence of these associated complicating conditions. Some of the most medically virtuous have succumbed early, while rascals have escaped. I will not say that only the good die young; but experience has convinced me that in this, as in many another, panel of life, virtue too often has to be its own and only reward.

Insults that alter the metabolism of normal individuals necessarily disturb that of diabetics. Indeed, since the metabolism of the latter is already impaired, it is more sensitive to such insults. Most infections aggravate diabetes. Just how they achieve their effects is not clear. It has been suggested that the impairment of carbohydrate metabolism is an expression of the injury reaction; but its severity does not seem to be, like that of the injury reaction, correlated with the gravity of the initial insult. This statement is made with reservations because neither the gravity of an infection nor the severity of a given diabetes can be quantitatively evaluated. Patients may, however, be driven into acidosis by a minor respiratory infection or furunculosis, yet tolerate a lobar pneumonia with little reaction. It is intriguing to inculpate the injury reaction rather than a specific susceptibility to infectious agents, since traumatic lesions, including surgical operations, have a comparable effect. There are even plausible reports of instances in which diabetes, either temporary or permanent, appears to have originated with an infection or injury that did not directly involve the pancreas or other endocrine glands.*

The impression that diabetes conduces to the incidence of and diminishes the resistance to infection is derived partly from traditions antedating insulin and the modern treatment of diabetes and partly from the failure to distinguish between the effects of the metabolic disorder per se and its consequences or associations. Before the advent of insulin, infections played a sinister rôle in diabetes because their aggravating effect on the metabolic disorder could not be combatted. For similar reasons the tolerance to trauma was equally poor. Since the nutrition of diabetics could not be maintained, they became victims of the same infections that multiply in famine areas, especially the great harvester, tuberculosis. Towards the end of the era of treatment by starvation and undernutrition it became apparent that pulmonary tuberculosis was a major, if not the greatest, single cause of diabetic mortality. The great incidence of chronic pyelonephritis in diabetic women and its probable causes have already been mentioned. A plea is entered for less instrumentation of this vulnerable urinary tract, especially the introduction of catheters, particularly inlying catheters inserted for the convenience of physicians, surgeons, and other attendants. Any part that has an inadequate blood supply is peculiarly susceptible to infection or trauma and less able to combat and repair injury. If the metabolic disorder is

* The most credible of these reports deals with self-limited or temporary diabetes. The incidence of temporary traumatic diabetes appears to have increased in recent years. This may be attributed to the greater number of accidents in which sudden deceleration with resultant concussion is involved. Injuries of the cranial contents are particularly prone to disturb carbohydrate metabolism.

properly controlled, the diabetic appears to be at no greater disadvantage than the non-diabetic with a similar degree of circulatory impairment. The patient who loses a leg after a minor accident to the toe may recover with a normal course after an infection of the hand or an abdominal operation. The patient with loss of sensation in his feet, whether diabetic or not, should not take off and put on his shoes and stockings in the dark nor warm or dry his feet in the oven.*

Although muscular exercise is not supported entirely by combustion of carbohydrate, as Myerhof and Hill once proposed, it does promote the preferential combustion of carbohydrate. The respiratory quotient rises even if the degree of activity is too small to increase the lactic acid or reduce the CO_2 of the plasma. The blood sugar does not rise, but hepatic glycogen falls, the supply of glucose by the liver keeping pace with its consumption by the muscles. These effects of exercise are not referable to a direct action of insulin; they are demonstrable in diabetic animals and people. In severely diabetic patients spontaneous combustion of carbohydrate is reduced to a minimum so that the effect of exercise is not easily demonstrable without insulin. Exercise, however, diminishes the insulin required by such subjects and, if insulin is prescribed without allowance for the effect of the exercise, may induce hypoglycemia. The administration of carbohydrate, or its consequence, hyperglycemia, also accelerates the combustion of carbohydrate, presumably by force of mass action.

Starvation or complete deprivation from preformed carbohydrate reduces the ability to burn carbohydrate and consequently diminishes the glycogen of the liver. Eventually the support of energy metabolism is taken over entirely by fat and protein. This involves a profound readjustment of the intermediary metabolism which cannot be immediately reversed; a state of starvation diabetes has been created. If, in this state, carbohydrate is taken, the blood sugar rises excessively and remains elevated unduly long; glucose will appear in the urine if sufficient carbohydrate is given. Not all the sugar given is excreted, however, by the normal person; the major portion is stored in the liver as glycogen. Only after the lapse of a certain amount of time after administration of enough carbohydrate does the metabolism return to its usual course. This inhibition of carbohydrate combustion, like

* A patient in the dispensary was found to have a deep chronic ulcer on his foot of which he had been unaware. He proved to have complete anesthesia of the feet and it was learned that he had a peculiar habit of always taking his shoes and stockings off and putting them on in the dark so that he never saw his feet. On a subsequent occasion, though he had been carefully warned, he fell asleep while his feet were in the oven where he had placed them in order to warm them and to dry his shoes. He suffered deep burns that ultimately required the amputation of one leg.

the accelerating effect of exercise, is not mediated by insulin; in fact, starvation retards the action of insulin. Diabetes mellitus does not confer protection against starvation diabetes; on the contrary, deprivation of carbohydrate aggravates the disability of diabetes mellitus. Anything that accelerates the consumption of the stores of carbohydrate in the body hastens the onset and exaggerates the severity of starvation diabetes. Adult men, even after prolonged fasting, do not develop enough ketosis to produce appreciable acidosis; women, on the other hand, become acidotic after a fast of 48 hours or less, apparently because they use carbohydrate less economically than males do. The pregnant woman, who is even more extravagant with sugar, is peculiarly sensitive to starvation. Children appear to be less tolerant than adults. Physical exercise, since it creates a preferential demand for carbohydrate, hastens the onset of starvation diabetes. At the end of a ten-mile walk before breakfast Douglas' respiratory quotient fell to near 0.70 and ketone bodies appeared in his urine. His associate, Courtice, who habitually ate more carbohydrate, was able to extend his morning walks somewhat further before the appearance of starvation phenomena. Both had high and prolonged glucose tolerance tests after their exercise.

Although diabetes mellitus does not decrease the amount of carbohydrate in the body, it does accelerate the exhaustion of the exogenous or preformed carbohydrate and is, therefore, a potent agent in the production of starvation phenomena. The incidence and intensity of glycosuria in diabetics during the course of the day was found by Kydd and Peters to be greatest before and after, especially after, breakfast. Glycosuria which occurs only after breakfast can occasionally be eliminated by having the patient take the breakfast fruit juice a half-hour or an hour before the remainder of the meal. When only short-acting regular insulin was available, one of the most serious therapeutic problems was the prevention of glycosuria during the sleeping hours when the patient was starving and physically inactive. Merely increasing the dose of insulin before supper or giving an extra dose before bed did not solve the problem. It tended, rather, to provoke or increase resistance to the morning dose of insulin.

Next to severe diabetes itself nothing more rapidly exhausts the stores of preformed carbohydrate in the body than insulin itself, when given without food. It accelerates the combustion of extrahepatic glycogen and the conversion of carbohydrate to fat at the expense of liver glycogen, and retards the replacement of the latter by carbohydrate derived from protein. So long as the supply of exogenous carbohydrate is adequate, insulin not only accelerates its combustion and conversion to fat, but also tends to preserve hepatic

glycogen. In the absence of an adequate supply of exogenous carbohydrate it conduces to hyperglycemia and exhaustion of liver glycogen. Hypoglycemia, with its drastic effects on the circulation and the central nervous system and the unhappy disorders of behavior it provokes, is of itself highly undesirable. If it is prolonged until preformed carbohydrate is exhausted, as soon as the action of the insulin is dissipated starvation phenomena appear. In the diabetic patient these phenomena assume an aggravated form: hyperglycemia, glycosuria, ketosis, and resistance to insulin may ensue.

The objective in the treatment of any chronic disease should be to enable the patient to enjoy as full and untrammelled a life as possible. Medicine should not be entirely negative and restrictive. It should especially avoid emptying life of the features that give enjoyment and a sense of accomplishment. The therapeutic regime should not make the patient conspicuous; his ailments are his own business. With insulin preparations that are now available the diabetic should be enabled to pursue a normal course of life with no lets or hindrances other than adherence to a diet, injection of insulin, and analysis of urine, except as these may be necessary to meet complicating or associated disorders. The patient with advanced arterial disease cannot profitably return to digging ditches. Since all the activities and vicissitudes of life influence the course of diabetes, no satisfactory therapeutic regime can be established or adjusted in a hospital. Hospitalization is necessary or desirable only for the management of complicating conditions or emergencies.

The diet should be adequate in all respects and adapted to the activities of the patient. The evil effects of undernutrition are more costly than extra food and insulin. Obesity should be avoided by all persons: It is especially contraindicated for diabetics because it imposes an extra load on the vulnerable circulation and feet and also aggravates the diabetes. This may be only because it increases caloric consumption. Reduction of weight decreases the severity of diabetes and may in some mild cases eliminate all symptoms and signs of the disorder. One gram of protein per kilogram of body weight per day provides a margin of safety against wastage of this essential food. There are no contra-indications to a more generous allowance. For extremely obese persons the quantity will have to be based on some compromise between the actual and the desirable weight if reduction is to be successful. Some limitation of carbohydrate is desirable, if only in the interests of economy. Highly sweetened desserts are particularly to be avoided. Since it is difficult to estimate their composition, small errors in the evalua-

tion of servings may make large differences in the carbohydrate intake. Enough should be provided to permit the selection of a suitable meal from food offered in the usual home or restaurant, including some starchy foods, fruit, and generous amounts of vegetables. This requires at least 100 gm. of carbohydrates. Unless reduction of weight is desired, the allowance should be more generous, 150 gm. or more. The remainder of the calories must be furnished by fat. This cannot be estimated, with the aid of dietary tables, according to size and presumed activity. Such tables are useful only for the calculation of a first approximation. Ultimately the adequacy of a diet must be established empirically by observation of weight and growth.

Even the most skillfully calculated diet will seldom fit perfectly. The patient should be directed, while adhering to the diet as precisely as possible, to keep a record of difficulties encountered. This bill of complaints should be presented to the physician at intervals. It should be considered not only from the standpoint of nutritive requirements, but equally from the standpoint of tastes, habits, personal, national, and religious prejudices, convenience, and pocketbook. For convenience diets are estimated and recorded in terms of grams of protein, carbohydrate and fat, and calories, proportioned to the patient's size and activities. They should be prescribed in terms of prepared foods and household measures which the patient should learn to visualize. Social intercourse which centers much about the table is impracticable if meals must always be negotiated through scales and graduates. The diet should be as varied as possible. Foods seldom appear raw and naked. Some knowledge of their preparation is helpful in the analysis of their treacherous coverings and adornments. The patient should be encouraged and helped in efforts at self-education in these matters. Without such interest and support, adherence to a prescribed regime cannot be expected. To place the full onus for infractions of dietary and other regulations upon the patient is an evasion of responsibility by the physician, whose prior assumption should be that the regime has been improperly devised. To upbraid the patient as if weaknesses were sins may destroy the frank relations that should prevail between physician and patient. To invoke fear is cruel. Lapses should be met with sympathy and understanding, their consequences explained with kindly reason. Self-righteousness on the part of the physician does not benefit the patient. An honest compromise, even though not altogether desirable, may be necessary. A little liberty is better than license.

Children require especial consideration because of their malleability and dependence. They should be given as much liberty as is compatible with

safety, not become so knit to parents that they are deprived of free association with other children in healthy, enjoyable activities. Parents should be counselled to avoid as far as possible punitive measures in the control of treatment lest fear and deception be fostered. The first thought must be that misbehavior arises from the outcry of natural instincts and that efforts must be directed to the discovery and removal of its causes.

Diets must be planned with consideration not only of the quantity, but also of the distribution of food throughout the day. Long intervals between meals should be avoided. A late evening feeding should be included, especially for patients who are receiving insulin, not only to protect them against nocturnal hypoglycemia, but also to provide for the gastronomic rites of guest friendship that are inescapable features of all nocturnal social activities. These evening snacks need not be austerely defined, though they should be measured with judgment. A prolonged evening occupied in enjoyable activity merits a little generous appreciation. Otherwise injudicious indulgence in cake and ice cream has saved many a boy or girl at a late dance from the embarrassment of a hypoglycemic reaction. Children should also have something to eat in the middle of the morning, not only to protect them from insulin reactions, but because a "middle of the morning lunch" has become a part of the accepted routine of schools. An afternoon feeding is usually also desirable since the majority of children eat something after school and the increased physical activity that comes with release from educational bondage facilitates the combustion of carbohydrate. Diabetic children should attend *parties,* not only diabetic parties. The soul of a children's party is ice cream, a well-standardized article of food which is not vicious just because it tastes good.

The normal or basic diet should be quantitatively constant. Regulation of diabetes by varying insulin with total disregard of diet entails large fluctuations of urine and blood sugar and danger of hypoglycemia. On the other hand, when activity, especially physical activity, is for any reason sharply increased, extra food containing carbohydrate should be taken in anticipation rather than for correction of hypoglycemia. When the routine of life involves predictable variations of activity, both insulin and diet may be varied to fit the schedule. For example, people may be roughly divided into three classes: seven-day routiners, those who increase physical activity over the week-end, and those who spend their week-ends avoiding activity. These differences of habit may require corresponding variation of insulin. Schedules of college students may involve more complicated, but still predictable, variations. The physician can aid the patient to learn to meet the ordinary

vicissitudes of life intelligently. Too close adherence to a fixed regime may be as injudicious as too much laxity.

As an index of the regulation of diabetes, qualitative analysis of the urine for sugar is usually sufficient. Such tests should be made by someone in the household, preferably the patient, at appropriate intervals during the day, since the incidence of glycosuria is as important as its presence. While a suitable regime is being sought, it may be necessary to test the urine four times a day, before each of the three chief meals and before retiring; but the number of analyses should be reduced as a proper schedule is worked out in order that the regime may be as little onerous as possible. Few patients require more than two tests a day, one before insulin and breakfast in the morning, the other either before the evening meal or before bed. Many require only one a day or one every other day. In this case they should be instructed to alternate morning and evening tests. If on some occasion the urine is found to contain large amounts of sugar, or if the patient feels indisposed or ill, even if the symptoms are not characteristic of diabetes, more frequent analyses should be made to ascertain whether the indisposition has adversely affected the diabetes. The ideal objective is continuous aglycosuria; but in severe or unstable diabetes this cannot be achieved without danger of hypoglycemic reactions. In such cases freedom from gross glycosuria for a reasonable part of the 24 hours, in a few even freedom from overt diabetic symptoms, polyuria and polydipsia, will have to suffice. Before accepting these compromises as inescapable, however, thoughtful search should be made for the most suitable arrangement of insulin and meals that will not distort life intolerably. These compromises should not include reduction of the diet below optimum requirements since this only substitutes for the diabetic disorder others that are more serious. The patient should be taught how to alter insulin to meet ordinary contingencies and to call the physician when in doubt or difficulty. The physician must be available and willing to respond to these calls. Advice over the telephone at the crucial moment may prevent admission to the hospital in acidosis.

Many of the expedients that contribute to the successful prescription and adjustment of insulin are implicit in the preceding discussion. The urine specimen before the noon meal* has the least significance, especially if the patient is receiving NPH or other mixtures of long-acting and short-acting insulin, because it is influenced by so many different factors: the long

* This and similar terms are used throughout this paper because they apply to the majority of subjects. For night workers and others who follow unusual schedules they must be translated to comparable times and intervals.

overnight fast, the residual effect of the long-acting insulin, the acute effect of the short-acting insulin, the morning meal and variable activity after the long night's rest. The urine before the evening meal shows the effects of both types of insulin, but is usually more indicative of the effect of the short-acting. The late evening and morning specimens are criteria of the long-acting insulin; but it must be recognized that increasing glycosuria in these last specimens may signify not that the patient received too little long-acting insulin, but that hypoglycemia occurred earlier in the course of the 24 hours.

Since carbohydrate and insulin cannot be administered continuously in proportions so balanced as to meet the changing metabolic requirements of life, it is impossible to keep the blood sugar of the diabetic patient within normal limits throughout the day without precipitating hypoglycemia at frequent intervals. If the urine is kept free from anything but traces of sugar for a considerable part of the day and night, there can be reasonable confidence that prolonged excessive hyperglycemia has been prevented. Some subjects, to be sure, especially among those with severe functional impairment of the kidneys, will maintain a high blood sugar while excreting little glucose in the urine. To attempt to regulate their diabetes in accordance with the blood sugar over any appreciable period is quite impracticable because it requires sampling of blood at frequent intervals. Some would challenge the utility of maintaining a normal blood sugar if it were feasible, since hyperglycemia promotes the oxidation of carbohydrate. Within limits this attitude is defensible; but, since glucose does not enter the mass of tissue cells freely by diffusion, hyperglycemia induces cellular dehydration. If on this account hyperglycemia is to be avoided, there is no reason why it should be less obnoxious at one time of day than another. The time-honored practice of regulating diet and insulin of a diabetic who is receiving long-acting insulin and feedings at intervals on the basis of blood drawn in the postabsorptive state cannot be logically defended. The blood sugar taken at this time on Tuesday is no precise indication of what it may have been Monday or is likely to be Wednesday.

Determination of the blood sugar yields information of the greatest importance in case of emergency when the immediate status of a patient must be ascertained in order that urgent corrective measures may be instituted.* The urine sugar is no index of the immediate status, but a

* When there is a question of hypoglycemia, blood for analysis should be obtained before treatment, if possible; but remedial measures need not be withheld until the blood sugar is known. Administration of unnecessary carbohydrate has little danger compared with the administration of unnecessary insulin.

summary record of the metabolic events since the preceding voiding. When difficulty is encountered in the regulation of insulin it may be valuable to secure a blood sugar at a particular time of day, more often to verify a suspected hypoglycemia than a hyperglycemia. Whether sugar is to be measured for this purpose or as a routine procedure it can have no relevance unless it is drawn under the living conditions of a patient. If the patient usually arises at 6:00 a.m. and takes long-acting insulin before breakfast at 6:30 or 7:00, a postabsorptive blood sugar taken in the doctor's office at 9:00 can give no relevant information. If, because the physician is diverted, the venepuncture is delayed until 10:00, the observation becomes even more meaningless. If a patient is suspected of hypoglycemia while at work or just after work in the late afternoon, little will be gained by having him stop work some afternoon to sit in the doctor's office till the witching hour arrives. Knowledge of the blood sugar is of the greatest importance when it is of any importance, but the information to be derived from it is highly specific. Perhaps it should be determined as frequently or more frequently than it now is, not only in diabetes; but it should be taken with careful consideration of its significance and interpreted in the same spirit.

It has already been admitted that, if it is an evil, a certain degree of glycosuria will have to be accepted in unstable diabetics as the lesser of two evils. When it can be eliminated without any serious disturbances of life by merely observing healthy moderation of diet, there is no reason for allowing it to persist. When glycosuria causes polyuria and nocturia that interrupt normal diurnal activities and sleep, it warrants treatment in behalf of comfort. Besides these symptoms betoken a certain degree of continuous dehydration. There is some argument whether intermediate grades of glycosuria should be tolerated. As the carbohydrate intake of the diabetic is gradually increased there is a considerable interval between the increment that first induces glycosuria and the increment that is completely excreted as glucose in the urine. In this interval the proportion of each additional increment that is utilized progressively diminishes. This is an example of the fact that hyperglycemia promotes combustion of carbohydrate and may be an argument for complacency towards moderate glycosuria. On the other hand, the cellular dehydration caused by hyperglycemia cannot be entirely disregarded. Whether glycosuria and hyperglycemia have other deleterious effects has not been definitely settled, but cannot yet be unequivocally denied. In women it seems to conduce to irritation, inflammation, and infection of the external genitalia which are prone to extend to the urinary tract with deplorable results.

The treatment of diabetic acidosis must be reserved for separate discussion.

Since diabetes is a disorder of metabolism it must be treated with the utmost consideration of the physiology and chemistry of metabolic processes and the effect of other vital activities upon these processes. Its management requires a radical reorganization of the whole conduct of life, usually for the duration of life and must, therefore, be planned and conducted with the most careful and sympathetic attention to the patient's habits, predilections, and activities. Interest must not be centered on the metabolic condition to the neglect of complications and associated disorders. Knowledge of the true nature of diabetes will be advanced by an open-minded study of the natural history of the disorder.

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NORMAL RANGE OF SERUM BUTANOL-EXTRACTABLE IODINE OF HUMAN ADULTS*

In the diagnosis of masked hyperthyroidism, measurement of the serum precipitable iodine (SPI) (frequently also called protein-bound iodine [PBI]) has gained significance. Since 1951 determination of thyroxine-like or butanol-extractable iodine (BEI) has been the routine procedure in this laboratory.[10] This chemical tool is more specific than the PBI in the diagnosis of thyroid dysfunction. Not only does the BEI correlate better than the PBI with the clinical state of patients who have received inorganic iodine,[5, 6, 7] but the range of serum BEI in euthyroid persons is narrower and more precise than the normal range for PBI. The upper limit of the normal range of BEI is more definite than that for the PBI. Data have been collected to define the limits of normal for serum BEIs of adults. A separate paper describes the range for BEIs of 70 young men in the University community.[19]

MATERIALS AND METHODS

The BEIs of 64 euthyroid men and women, doctors, laboratory personnel, medical students and their friends, have been measured by the procedure already described.[10] There were 24 males and 40 females between the ages of 22 and 65 years. These volunteers were euthyroid and otherwise in normal health.

The validity of the maximum figure needed more confirmation than could be derived from data on only 64 euthyroid adults. Therefore, all of the BEIs between 5.0 and 8.0 gamma per cent which were determined between January 1, 1953 and July 1, 1954 were reviewed. Three hundred and thirty-eight patients in the clinics, in the New Haven Hospital, and in the Memorial unit of the Grace-New Haven Community Hospital had BEIs between 5.0 and 8.0 gamma per cent. Of these, only patients for whom it was possible to obtain clinical data subsequent to the measurements of BEI were included. Hyperthyroid patients under treatment were also excluded because this investigation pertained to the height of the BEI in relation to euthyroidism or hyperthyroidism before treatment. No children or adolescents were included.[5, 6, 18]

This left 284 subjects whose records were examined for evaluation of thyroid activity. One hundred and thirteen of the 284 were excluded because of paucity of clinical

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information or because the BEIs were open to question. The determination is affected in the patients who have received iodine-containing dyes for roentgenography,[17] mercurials,[20] desiccated thyroid,[10, 25, 30] cortisone, estrogens,[9] or multiple blood transfusions, or because pregnancy,[5, 18] thyroiditis, or brain tumors[21] complicate the picture. Also excluded were patients with liver disease[12] or with fever in acute infections.[29] Omission

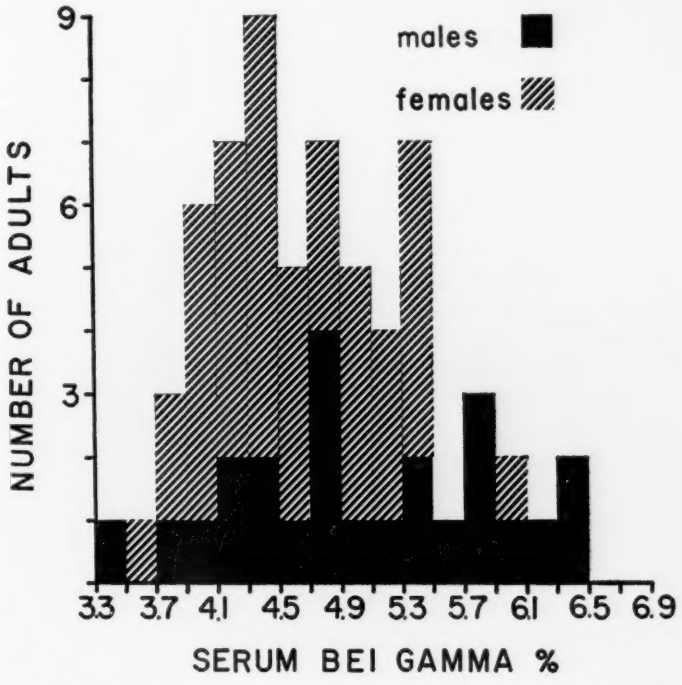

FIG. 1. Serum BEIs of 64 adults between 22 and 65 years of age, 40 females and 24 males.

of these last patients does not alter the data significantly, but their inclusion is not justified because of their clinical conditions. BEIs were above 6.4 gamma per cent in only four of the persons with liver disease and in three with febrile reactions. In 171 patients sufficient data were available to establish thyroid status. There were 132 females and 39 males 21 to 92 years of age. Ninety-four of the 171 patients were over 50 years old.

These 171 patients were classified with respect to the clinical symptoms at the time the BEI was measured and also in regard to the subsequent course. The persons judged euthyroid were separated into groups in relation to the most outstanding symptoms or signs. The BEIs of 70 patients with cardiovascular symptoms are represented in Figure 2 by rectangles with crossed diagonals. Of these 70, 47 had cardio-

vascular disorders such as tachycardia, auricular fibrillation, hypertension, arteriosclerotic heart disease, dyspnea, orthopnea, asthma or allergic reactions, rheumatic heart disease, mitral stenosis, or aortic insufficiency. Twenty-three not only had some of the above symptoms, but enlargement of or nodules in the thyroid or a history of previous thyroidectomy. In addition to the patients with cardiovascular symptoms were 21 persons with nodular or nontoxic goiters or a history of previous thyroidectomy, 15 psychoneurotic patients with anxiety or tension, 15 with menstrual irregularities, 11 with malnutrition, malignancy, tachycardia, or low grade fever, and 13 whose BEIs had been measured because hypoactivity of the thyroid was questioned. The BEIs of the patients in these last five groups are not differentiated in Figure 2 because their distribution is not distinctive.

RESULTS

The BEIs of the 64 euthyroid adults are shown in Figure 1. The mean is 4.7 and the S.D. is ± 0.72 gamma per cent. The data, as might be expected, are not symmetrically distributed. Considering the two determined BEIs of 6.4 gamma per cent a reasonable estimate of the range for serum BEIs of euthyroid adults is 3.2 to 6.4 gamma per cent. There was no apparent relationship between age and the BEIs of these 64 persons and no difference between males and females.

Of the 171 patients with BEIs between 5.0 and 8.0 gamma per cent who were classified with respect to the clinical symptoms, 148 were judged euthyroid and 23 hyperthyroid. In Figure 2, 145 BEIs of the 148 patients who on clinical evidence were judged euthyroid fell within the inclusive range of 5.0 to 6.4 gamma per cent. Three patients with multiple cardiovascular symptoms had BEIs of 6.7 and 6.8 gamma per cent. From the clinical history the man with the BEI of 6.8 gamma per cent, severe hypertension, and cardiomegaly, may have had an overactive thyroid at the time the BEI was measured. Since then he has died and no autopsy was obtained.

In Figure 2 are 23 patients who were judged hyperthyroid and who are designated by solid rectangles. All 23 had BEIs between 6.5 and 8.0 gamma per cent.

Data on these 171 patients support the value of 6.4 gamma per cent as the upper limit for euthyroid persons. The three BEIs of euthyroid individuals above this limit exceeded 6.4 only by 0.3, and 0.4 gamma per cent. Data obtained from random BEIs measured during the first six months of 1953 revealed that the S.D. of the mean of duplicate determinations at a level of 6.0 to 8.0 gamma per cent was ± 0.42 gamma per cent. None of these three BEIs varied by as much as twice the S.D. of the mean from 6.4 gamma per cent, the maximum of the normal range.

DISCUSSION

The above data are presented to encourage recognition of hyperthyroidism in those persons with equivocal symptoms of hyperthyroidism and with BEIs slightly above 6.4 gamma per cent. Use of the frequently cited normal range for PBI of 4.0 to 8.0 gamma per cent[1-5, 11, 18, 22-24, 26-28] does not permit

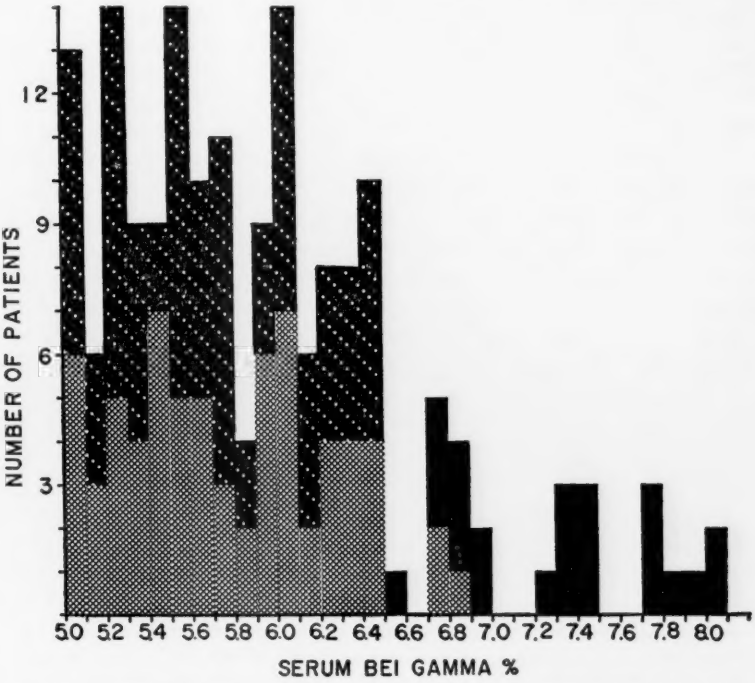

FIG. 2. Serum BEIs between 5.0 and 8.0 gamma per cent of 171 adults aged 21 to 92 years. One hundred and forty-eight adults in whom by clinical criteria thyroid activity was normal are represented by rectangles with white dots and by rectangles with crossed diagonal lines. The latter designate the 70 patients with cariovascular symptoms. Solid rectangles represent 23 patients who were clinically hyperthyroid. Note that the range for BEI is narrower than and the scale twice that of Figure 1.

recognition of hyperthyroidism in certain patients with little cardiovascular reserve in whom remission of thyroid activity may well restore the cardiovascular system to a safe reserve. Our own laboratory is not blameless in publishing SPI values which support a normal PBI range of 4.0 to 8.0 gamma per cent.[18] Of 80 euthyroid adults the SPIs of two persons were 7.5 and 7.8 gamma per cent. The range for SPI's in hyperthyroidism was cited

as 8.0 or more gamma per cent with four exceptions; the four exceptional hyperthyroid patients had SPIs of 7.1, 7.4, 7.8, and 7.8 gamma per cent. If the standard amount, 0.5 gamma per cent, by which the SPI exceeds the BEI[18] is subtracted, the two lower SPIs would correspond to BEIs of 6.6 and 6.9 gamma per cent. Both of these patients improved when they received medical treatment for thyroid overactivity. In the present series a diabetic hypertensive woman, aged 68, with a BEI of 6.7 gamma per cent, a woman of 58 admitted for mitral valvulotomy with two successive BEIs of 6.5 and 6.8 gamma per cent, a 48-year-old woman with tachycardia of 130, tremor, suggestive lid-lag and goiter, and a BEI of 7.4 gamma per cent improved clinically when treated with Lugol's solution alone or combined with propylthiouracil. Two males, aged 21 and 30 years, with BEIs of 7.3 and 6.7 gamma per cent respectively, improved clinically after medical treatment for hyperthyroidism.[19] Utilization of a euthyroid range for PBI up to 8.0 gamma per cent would not have revealed the overactivity of the thyroid in the three women described above, in the four patients mentioned, or in the two university students included in another paper. In a previous paper[18] 7.1 gamma per cent appeared to be the statistical maximum for the SPIs of euthyroid adults. Subtraction of the average difference between the SPI and BEI would correspond to a BEI of 6.6 gamma per cent as the upper limit of the euthyroid range, a value only 0.2 gamma per cent in excess of the maximum derived from the BEIs of the euthyroid adults included in this paper.

In accepting 6.4 gamma per cent as the top of the BEI range for euthyroid adults certain limitations must be considered. Values above 6.4 gamma per cent do not connote hyperthyroidism in either the pregnant woman[5, 15] or in the child younger than ten years of age.[8] The newer contrast media used for intravenous pyelograms or for arteriograms, angiograms, cholecystograms, salpingograms, etc. may elevate the BEI for months. "Compound diodrast" invalidates BEIs, probably for at least two months, much longer than the simple diodrast used some years ago.[17] Compounds of iodine and thiouracil give falsely high values; itrumil excluded measurement of the BEI of one patient until six weeks after cessation of the drug. Iodine-containing medication such as iodoquinone, fluoroquine, or iodoform packs has at times seemed to elevate the BEI. The fever of acute infection[20] or severe liver dysfunction[13] may affect the BEI so that after the acute phase BEIs should be repeated to confirm an abnormal observation obtained during acute illness. For example, a woman with pneumococcal meningitis had a normal BEI of 5.7 gamma per cent when she was afebrile eight days after her temperature had been 105° F. and her BEI 2.7 gamma per cent.

CONCLUSIONS

The range for serum BEIs of 64 euthyroid adults between the ages of 22 and 65 years was from 3.2 to 6.4 gamma per cent. There was no relationship between age and the BEI.

One hundred and seventy-one patients between the ages of 21 and 92 years in whom thyroid dysfunction was suspected and with BEIs between 5.0 and 8.0 gamma per cent were classified by clinical criteria into euthyroid and hyperthyroid groups. One hundred and forty-five of the 148 euthyroid adults had BEIs within the inclusive values of 5.0 to 6.4 gamma per cent. Twenty-three persons judged hyperthyroid had BEIs of 6.5 to 8.0 gamma per cent.

From the data the upper limit of serum BEI for euthyroid adults is 6.4 gamma per cent.

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SERUM BUTANOL-EXTRACTABLE IODINES OF MALE UNIVERSITY STUDENTS

Young men and women sometimes present a variety of disorders such as fatigue, obesity, and vasomotor disturbances which cannot be related to definite functional or organic abnormalities. In evaluation of thyroid activity of these patients, measurement of the serum BEI, butanol-extractable iodine,[3] has been of limited value because the normal range for BEI of adolescents and young people has not been defined. Euthyroid children have a higher normal range for BEI (4.5 to 7.3 gamma per cent)[1] than adults (3.2 to 6.4 gamma per cent).[3] Data on the BEIs of 70 male university students between the ages of 18 and 31 years have been examined in comparison with the values for euthyroid adults. However, the BEIs of the students are not those of men in whom euthyroidism was claimed but of men in whom the various signs and symptoms necessitated that thyroid dysfunction be excluded.

All of these 70 students had psychogenic disorders such as fatigue, sleepiness, difficulty in studying, under- or overweight, tachycardia, intolerance to cold, moist cold or warm palms, dry skin, a history of low basal metabolic rate or thyroid medication. In none of the group was enlargement of the thyroid or the presence of a thyroid nodule observed. No demonstrable cause for the signs and symptoms could be found by means of physical examination, chest films, complete blood count, hematocrit, sedimentation rate, urine analysis, and blood sugar determination. Except for two persons from Honolulu and Australia the homes of the young men were in 22 different states of the U.S.A. distributed between Maine and California.

RESULTS

Two fatigued, tense students, one (G.P.) with a slight tremor, the other (C.I.) with insomnia, a pulse rate of 120 to 130, and labile blood pressures between 160/70 and 130/70 improved clinically after receiving 10 drops of Lugol's solution three times a day for about two weeks. These clinical responses to Lugol's solution imply that the thyroids of these men were

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slightly overactive and their initial BEIs of 6.7 and 7.3 gamma per cent cannot be included in the normal range. After about two weeks of therapy with iodine solution the BEIs had decreased to 5.6 and 4.5 gamma per cent.

Five men between the ages of 20 and 31 years had BEIs of 2.6, 2.8, 2.8, 3.0, and 3.1 gamma per cent. These five men had various physical abnormalities. Two were overweight, two were underweight, two were anemic, two were sensitive to cold, one had a fine tremor, Jacksonian epilepsy, and allergic rhinitis. Two had taken thyroid previously but had stopped this medication at least six weeks before the BEI was measured. In view of their symptoms and serum BEIs, four of the five were given desiccated thyroid in amounts varying between 60 and 180 mg. daily. On this therapy, three have improved clinically and the fourth, an obese man, lost weight on only 60 mg. daily. The response of the fifth student to thyroid medication is not known. Considering the clinical responses to such therapy of four of these men with serum BEIs below the minimum for normal adults, none of the five low BEIs can justifiably be included in the normal range.

After exclusion on clinical grounds of the two high and of the five low BEIs, those of the remaining 63 students were charted (Fig. 1) and examined statistically. The mean and S.D. for these 63 were 4.7 and ± 0.75 gamma per cent and from the 95 per cent limits the range would be 3.2 to 6.2 gamma per cent. These values agree precisely with those for normal adult men and women.[3]

DISCUSSION

Interpretation of the data in Figure 1 yields information in regard to the BEIs of young men. The BEIs of 6.7 and 7.3 gamma per cent of the two students who improved clinically after receiving Lugol's solution would be classified above the euthyroid range; the upper limit of normal for adults, 6.4 gamma per cent, fits men of this age better than 7.3 gamma per cent, the upper limit for euthyroid children younger than ten years of age. The lower range for normal BEIs of young men is not so clear. Four students with BEIs below 3.2 gamma per cent improved clinically when given desiccated thyroid but this therapy was not given a clinical trial in any men with higher BEIs. The values for BEIs between the normal minima for children and adults might denote either underactivity of the thyroid or normal thyroid function simultaneous with increased peripheral utilization in active young men allowing themselves a minimum of sleep. Further investigation of the lower limit for the BEI range of euthyroid young men is needed.

Evaluation of the normal range for BEIs of young men is not aided by measurement of the basal metabolic rate, a test which has recognized short-

comings. For example, eight students with a history either of a low basal metabolic rate or of receiving desiccated thyroid at a time more than six weeks prior to BEI measurement had BEIs between 4.4 and 5.8 gamma per cent, euthyroid values even by standards for young children. These data add to the clinical opinion that desiccated thyroid administration on the basis of basal metabolic rate without additional proof of hypothyroidism

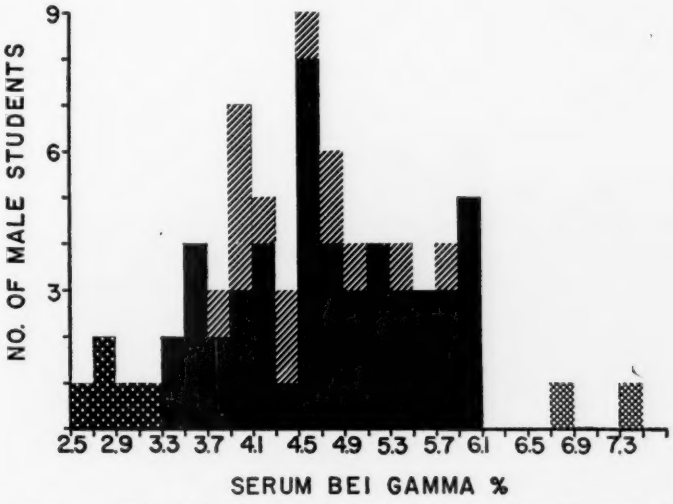

FIG. 1. Serum BEIs of 70 male university students between 18 and 31 years of age. Solid rectangles represent young men without dysfunction of the thyroid. Rectangles with diagonal lines represent men with histories of low basal metabolic rates or of medication with desiccated thyroid. Rectangles with dots represent five young men who by clinical evaluation were hypothyroid. Rectangles with crossed diagonals represent two men who by clinical evaluation were hyperthyroid.

is not justified. Of course the value for a recent serum BEI does not give information about clinical symptoms or need for desiccated thyroid years previously. Clinical confirmation of thyroid dysfunction in this age group is difficult due to complications. The symptoms of the 63 young men without recognized thyroid dysfunction did not differ clinically from those which have been described for the seven students excluded from a euthyroid status. Thirty of the 63 reported fatigue of at least three months' duration. Eight had tachycardia, palpitation, elevated or labile blood pressure, and three were anxious and tense. Two of the 63 had duodenal ulcers. Four were underweight or had lost weight; seven had gained weight or were

overweight. Fourteen either had a history of a low B.M.R. or of having taken desiccated thyroid.

Since these students at Yale have homes in many parts of the country, areas near the seacoast or areas deficient in iodine of sea water, the BEIs were charted in relation to the geographical distribution of their homes for at least the past three years. Seventeen young men from California, Texas, Mexico, Oregon, Idaho, Washington, Minnesota, and Colorado had BEIs between 3.0 and 6.0 gamma per cent. Thirty-seven men from the Atlantic seacoast had BEIs between 2.6 and 7.3 gamma per cent, while two from this area had clinical symptoms of hypothyroidism and two of hyperthyroidism. Sixteen subjects who came from states in the middle of the country had BEIs between 2.8 and 5.8 gamma per cent. No distinct pattern of the concentration of the BEI correlates with the geographical area from which the individual came.

CONCLUSION

Of 70 male university students, with vague symptoms and varying in age from 18 to 31 years, two with BEIs of 6.7 and 7.3 gamma per cent were judged hyperthyroid because they improved clinically after receiving Lugol's solution. Four of five individuals with BEIs below 3.2 gamma per cent were judged hypothyroid because they improved clinically after taking 60 to 180 mg. of desiccated thyroid daily. The other 63 young men had BEIs distributed similarly and throughout the same normal range, 3.2 to 6.4 gamma per cent, as euthyroid adults.

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HISTORICAL AND GEOGRAPHICAL ASPECTS OF THE EPIDEMIOLOGY OF POLIOMYELITIS*

Some of us who have been concerned with the epidemiology of poliomyelitis for a decade or more have finally come to realize that the behavior of this disease differs in different places and has differed in different times. Prior to this realization vain attempts had been made to make this disease conform in its behavior to a universal pattern, which could be presented as simple truths in a concise and digestible manner in textbooks of medicine and pediatrics. Today it is borne in upon us that as in many other situations, each truth can be related only to the circumstances—or the setting in which it has been observed. Thus the behavior of poliomyelitis under different environmental or sociological conditions varies from that of an "endemic disease" in infants as in Cairo, Egypt, to an "epidemic disease" of school children or young adults such as one might find in a North American city.† Similarly the *infantile paralysis* of our grandparents' time is somewhat different from our polio of today, in the creation of which even the impact of the newspapers has been felt. In fact, ideas about poliomyelitis have had to be constantly revised and probably will continue to undergo revision. This should explain, perhaps, the title chosen for this lecture: *Historical and Geographical Aspects of the Epidemiology of Poliomyelitis,* and in this choice of a title I have shown no great originality, for I have merely taken a page from a well-known book by August Hirsch: *"Handbuch der historisch-geographischen Pathologie,"* first published almost a hundred years ago.[11]

Historical. Acute paralysis in infants as a disease entity goes back to biblical times, perhaps earlier, but it does not seem to have attracted the attention of physicians prior to the late eighteenth century. Nevertheless, by the early nineteenth century, infantile paralysis was widespread geographically, for the earliest clinical descriptions of it came from several diverse areas: England, 1795; Italy, 1813; India, 1823; and U.S.A., 1830.[20]

* The Fifth Annual Don W. Gudakunst Memorial Lecture presented on May 25, 1954 at the School of Public Health, University of Michigan, Ann Arbor, Michigan.

† The terms: *endemic* disease and *epidemic* disease here have been used in an arbitrary sense; endemic meaning frequent sporadic cases without epidemics, and epidemic meaning both sporadic cases and periodic epidemics.

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The disease was regarded at that time as ubiquitous and due either to "teething," "foul bowels," or to a "fever." There was no mention of contagion, or of epidemics, nor was the condition regarded as a medical problem of any magnitude. As to how common or how prevalent endemic poliomyelitis was during the greater part of the nineteenth century, this is a matter for speculation; but Heine,[9] an orthopedic surgeon in Germany, was able to collect a series of 30 late paralytic cases and publish a monograph in 1840 describing them. And coincidentally in this country, "orthopaedic surgeons" of the 1830's made occasional mention, without comment as to rarity, of cases of club foot due to paralysis, acquired in infancy as a result of teething or fever.[20] Apparently then, the general situation in our grandparents' or great-grandparents' time was similar to that which can be found today in certain countries where substandard sanitary conditions exist and where this disease in endemic, limited to infants, where epidemics in local inhabitants are unknown, and where it is not regarded as a problem of major importance.

The first indication of anything which might be called an epidemic appears in a report by Sir Charles Bell, the anatomist and experimenter[2] in 1836. He stated that a "group of cases" of paralysis in children had occurred in an isolated community, namely, on the Island of St. Helena. Dr. Bell believed that the situation "deserved looking into." In the same year, Badham in England reported four cases of acute paralysis which had occurred more or less simultaneously in the small community of Worksop.[3] He also regarded this episode as ominous, although he hesitated to contend that any real element of contagion existed in this situation, perhaps because contemporary authorities were not familiar or sympathetic with any such idea. It was the reading of Badham's paper that prompted Heine, in Germany, to assemble his series of some 30 late paralytic cases. Here he firmly states his belief that the trouble is in the spinal cord, but he did not mention the epidemic character of the disease. A few years later there appeared Colmer's report, which has often been quoted, though erroneously interpreted as being the first description of contagion in poliomyelitis. This item which was printed in the American Journal of Medical Science in 1843[8] is quoted herewith:

Medical Notes. By George Colmer. *Paralysis in Teething Children.* Whilst on a visit to the parish of West Feliciana, La., in the fall of 1841, my attention was called to a child about a year old, then slowly recovering from an attack of hemiplegia. The parents (who were people of intelligence and unquestionable veracity), told me that eight or ten other cases of either hemiplegia or paraplegia, had occurred during the preceding three or four months within a few miles of their residence, all of which had either completely recovered, or were decidedly improving. The little sufferers were

invariably under two years of age, and the cause seemed to be the same in all—namely, *teething.*

It seems clear that Dr. Colmer did not regard this situation as an epidemic of an infectious disease.

But in spite of these limited and scattered outbreaks in the mid-nineteenth century, poliomyelitis was not destined to become quickly recognized as a disease entity with infectious or epidemic potentialities. Not until 1868 were groups of cases reported in Norway[4]; and it was still later when the pediatrician, Medin,[17] and others described outbreaks in Sweden, that the epidemic character of poliomyelitis began to be taken seriously. Furthermore, recognition of this new trend, if so it can be called, was for some 25 years limited largely to northern Europe, for textbooks articles on infantile paralysis which appeared in this country during the 1870's and 1880's fail to make mention of it, other than to state that various infectious diseases might be the immediate causal antecedent of paralysis in childhood.[18] However, by 1895 ideas about the disease had entered a new phase; its epidemic potentialities, were recognized, and it had temporarily acquired a new name: Heine-Medin Disease.*

It was after the turn of the century that Medin's pupil, Wickman, in Sweden,[30] began his classic work. This challenged the emphasis upon paralysis, which had previously dominated all thought in this disease, and became the basis of more modern epidemiological concepts, with an appreciation of its infectious nature, its spread through human association, or possibly through the agency of milk, and the importance of mild, nonparalytic cases as carriers. Not only did Wickman recognize the mild cases, but he recognized their epidemiological significance—for by including them in his epidemiological studies he was better able to trace the comings and goings of the disease in small villages and communities than had his predecessors. Indeed, he had some inkling that nonparalytic poliomyelitis was actually the "typical form" of the disease with paralysis as a "sort of complication," albeit a spectacular one. It was *clinical epidemiology* at its best, in spite of the fact that he was working in the dark as far as knowledge of the etiological agent was concerned, for all his field work was done prior to the discovery of the virus of poliomyelitis by Landsteiner and Popper in 1908.[16]

If one were to continue to search for names (appropriate in 1910) to add to the title of this disease, one could compose the following: Heine-Medin-Wickman-Landsteiner Disease.

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* In the meantime other names had crept in, some of which today sound singularly cumbersome and inept. Among these were: acute fatty atrophic paralysis; essential paralysis of childhood; tephromyelitis, etc.

But let us not forget that a decade before Wickman, Caverly, a physician in Vermont, first described nonparalytic poliomyelitis.[8] The basis of his description was an epidemic in Otter Creek Valley near Rutland, Vermont. It reminds us that only when fairly large numbers of cases were seen together as in an epidemic that observations on the variegated clinical picture of this disease were at last made possible.

Since 1900, and with increasing rapidity, epidemics of poliomyelitis appeared in Europe, North America, and elsewhere. Areas and approximate dates where and when this transition began are: Scandinavia, 1860-1890; western Europe, 1880-1890; the northeastern section of the U.S.A. in the 1890's; and the southeastern section two decades later. From a comparative curiosity, infantile paralysis had become a periodic scourge. And once epidemics of poliomyelitis have begun to appear in a given region, the pattern has not been reversible. Furthermore, there has generally been a sharp or gradual progressive increase in prevalence, as measured over ten-year periods. In some countries the annual number of cases has risen over the past 20 years, as in the U.S.A., Canada, and France; in others, the number of cases has maintained itself at fairly high levels during this period with widely irregular swings.

Still more recently, i.e., within the past 20 years, has this epidemic evolution of the disease come to pass in tropical and semi-tropical areas such as Puerto Rico, Hawaii, Malta, El Salvador, and the Island of Mauritius. In keeping with the idea that poliomyelitis has not usually been considered to be a disease of the tropics, the thought usually was, when these tropical epidemics appeared, that a "new disease," or at least a new strain of virus, had suddenly been introduced into the community. This may well have been the case in some places; but, on the other hand, many tropical populations had not been previously aware of the endemic character of their own local poliomyelitis or of how common it was. It certain places of this type, such as the Philippine Islands, the native disease only came to the surface when groups of foreigners, such as U. S. soldiers, entered the country as "susceptible immigrants," and there these military populations acquired poliomyelitis at far higher rates than those reported locally in native civilians and at far higher rates than would have been expected in their homelands. This was noted in the Philippines as early as 1936 and subsequently,[10] i.e., before, during, and after World War II.[38] Documented examples have also occurred in Cairo, Egypt[21]; in India[16]; and in French Morocco.[23]

Another evolutionary change which followed the advent of epidemics has been a gradual shift in *age incidence*. During the "pre-epidemic era," of the

nineteenth century and before, poliomyelitis seems to have been almost entirely a disease of infants under five years of age. Indeed, one might term the *primitive form* of poliomyelitis as the true "infantile paralysis." This concentration of 90 per cent of the paralytic cases within the infantile age group is seldom seen in north Europe or Scandinavia today, or in the U.S.A., Canada, or Australia; for, whereas 40 or 50 years ago maximum attack rates were nearly always recorded in the 0-4 age group, this soon shifted to the 5-9 age group, or, as in Sweden at present, to even older age groups.[19]

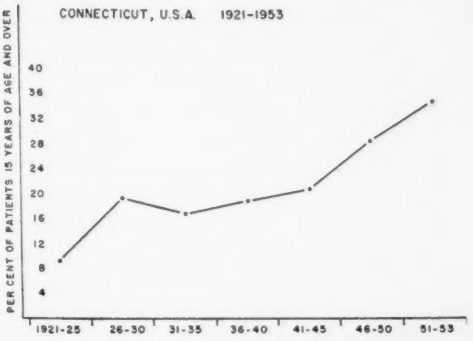

FIG. 1. The per cent of cases of poliomyelitis which occurred in Connecticut patients who were over the age of 15. Annual lists of such cases have been averaged for five-year periods, for the period 1921-1953, inclusive. A progressive rise on this percentage is apparent. (Data obtained through the kindness of Dr. Mila Rindge, Bureau of Preventable Disease, State Department of Health, Connecticut.)

It is obvious that the factors which wrought this change were multiple: The composition of the population as far as age groups are concerned, the curtailment in the size of the families, and perhaps others. But important among these factors has been a shift in the opportunities for exposure early in life. It had long been appreciated that the age incidence of poliomyelitis, like that of measles a generation ago, varied with the concentration of population; i.e., the more densely populated the community, the younger the age of those affected. It was not until about 1930 that it became evident that urban cases were becoming older and that a general or world-wide trend towards an *increase in the age* of poliomyelitis patients, was taking place. Burnet[8] was a pioneer in placing emphasis on the epidemiological significance of this trend. Today in the northeastern part of the United States as many as 35 per cent of the patients may be 15 years of age or older (see Fig. 1). This is a phenomenon of *epidemic* as opposed to *endemic* poliomyelitis and, as adult cases are apt to be more severe, with this rising age incidence cases are more readily diagnosed and reported, and therefore attack rates increase accordingly. For these reasons alone, it is easy to see how the *epidemic* form of poliomyelitis has attracted much more attention, more alarm, and more study than has the endemic disease.

For a number of years this shift from an endemic to an epidemic disease, from an infantile to a childhood disease was without explanation.[12] But most of the remainder of this article will be concerned with a review of evidence as to what has been responsible for this evolution. It will also be concerned with newer measurements on which the evidence is based and which now have made it possible to estimate the *immunity status* against poliomyelitis existing within certain populations and communities.

Geographical. We have already mentioned that poliomyelitis like many another disease spread by human association (i.e., like measles and mumps) is world-wide in distribution, and there is no reason to believe that there is any part of the inhabited globe to which the disease will not penetrate. Nevertheless, the prevalence of reported cases of poliomyelitis varies enormously in different places, and it can be cautiously stated, as of 1954, that those countries which pride themselves on having advanced standards of sanitation and low infant mortality rates have the highest poliomyelitis rates. Furthermore, the epidemiological behavior, and the clinical behavior of poliomyelitis, vary in other ways in different places as they have in different times: from that of extreme endemicity with low rates and a disease confined to infants to that of recurrent epidemics with involvement of older age groups and high rates when computed over a ten-year period. As to the mechanisms of spread involved, granted that poliomyelitis is an infectious disease which is easily transmitted from person to person, one may well ask about current ideas as to the type of human association needed for transmittal. Although one cannot be dogmatic on this point, it is my belief that most cases of poliomyelitis, though not necessarily all, are acquired as a result of a susceptible person coming in contact with an individual in the infectious or infective stage, regardless as to whether he or she be suffering from an overt, (paralytic or nonparalytic) *abortive case,* or an *inapparent* infection. There is some evidence to believe the severe paralytic cases are the most dangerous spreaders of infection but it is the inapparent form of infection which makes up the bulk of so-called "healthy carriers."

As to the nature of the infectious stage, it appears that early in the disease the virus appears simultaneously in the blood stream, the throat, and the intestinal tract. Subsequently it persists for varying periods of times in the intestinal tract alone. Recognizing these two possible portals of exit for the virus, it is easy to imagine that children can readily contaminate their playmates and their immediate environment. That contamination of the environment also plays some part seems likely, for reasons to be mentioned presently. In any event poliomyelitis is apparently one of those

diseases which, like measles, is ever ready to spread into a susceptible population; and it will do so whenever the virus has access (during the appropriate season) to such a susceptible population, unless the mechanism or spread is removed by artificial or special circumstances. Today, there are few communities no matter how small or remote, how sanitary, or how modern, which can hope to escape periodic contact with this virus forever, and, if the inter-epidemic period is long, the risk of a potentially severe epidemic increases annually, as a population composed of susceptible children, adolescents, and even young adults is built up. This susceptible population therefore represents a kind of vacuum of growing size into which the virus may surge when the opportunity offers, and the older the susceptibles are, the more severe is the epidemic likely to be. Few more graphic examples of this are at hand than that of the poliomyelitis epidemic among Eskimos in the Canadian Arctic in 1948-49,[27] and again in 1953.[14] Here in the fall of 1948 the disease was first introduced into settlements along the west coast of Hudson's Bay and spread rapidly northwards, reaching the remote settlement of Chesterfield Inlet in mid-winter, where the epidemic involved almost 20 per cent of the entire population with paralytic disease, attacking all age groups, the lowest clinical attack rate being in the infants![27] The same situation was encountered in another village in 1953.[14] This fact reminds us that, as in measles, the age distribution of cases of poliomyelitis within a community or population can also be taken as an indication of the state of immunity of that population, which in the case of these Eskimos must have been exceedingly low.

Today, however, measurements besides those based on age specific case rates can be made which may enable the epidemiologist to evaluate the immune status of a given population in advance of an epidemic. This has been done in many areas by determining the per cent of those healthy individuals in the chosen population who possess antibodies capable of neutralizing one or more types of poliomyelitis virus. Such neutralizing antibodies persist after infection probably for life and indicate whether the individual who possesses them has been infected either in the recent or distant past. In other words, although the presence of antibodies and immunity is not one and the same thing, neutralizing antibodies represent the footprints of disease, whether they be clear or faint, which indicate that an infection has passed that way. And again, although the significance of various antibodies differs, a general pattern can be found which allows one to use this method to determine roughly the "immune status" of a given population not only to poliomyelitis but to a variety of different infections.[28]

Originally such surveys on the serological epidemiology of poliomyelitis were carried out in various parts of the world with very crude and ex-

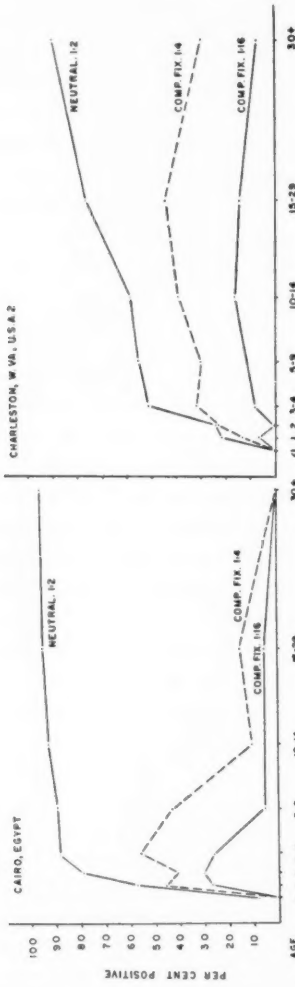

FIG. 2. Age distributional antibody patterns to Type 2 poliomyelitis virus in the populations of two cities (Cairo, Egypt, and Charleston, W. Va., U.S.A.) which differ widely as far as local environmental conditions are concerned. The neutralization tests (done with a serum dilution of 1:2) indicate a rapid rise in infancy of these antibodies in Cairo (on the left), as compared with Charleston (on the right). The complement fixation tests done with two serum dilutions 1:4 and 1:6 indicate a concentration of Type 2 poliomyelitis infections in the age group under 10 in Cairo, which is not apparent in Charleston. (Cairo data from Paul *et al.*[28]; Charleston data obtained through the kindness of Drs. Mary Walton and J. L. Melnick.)

pensive tests in monkeys, later in mice, but today, thanks to discoveries by Enders and his co-workers, in tissue cultures. This special approach has allowed measurements on poliomyelitis antibodies to be made on a large

scale. The serological survey can now take its place along with tuberculin test surveys or Schick test surveys. In other words, it aims to differentiate the immunes from the susceptibles within a given population.

This survey method has been most useful in tracing the presence of infection in remote areas. Thus in two remote Eskimo communities, North Alaska,[25] and Baffin Island,[7] it has been found, as one might have expected, that there was a dearth of neutralizing antibodies of all three types of poliomyelitis in over 95 per cent of the young adult population. In contrast, with-

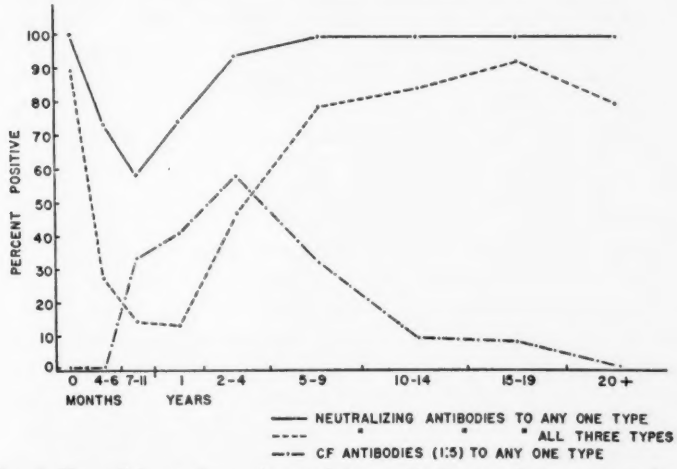

FIG. 3. Neutralizing and complement-fixing antibodies to all three types of poliomyelitis virus as determined in a group of the juveniles and adolescents from Northwest Africa. Heavy infection in early infancy is apparent with a good deal of such infection occurring within a very brief period after the loss of maternal neutralizing antibody. After the age of 10 years, the curve for complement-fixing antibodies reveals very little evidence of reinfection or new infections in this population. (Data from Paul and Horstmann.[22])

in certain crowded areas both urban and semi-urban, the situation may be quite the opposite, for surveys made in areas with substandard sanitation, many of them in the tropics, have shown that almost all healthy children over the age of four or five years possess Type 2 neutralizing antibodies, indicating that they had already been infected with this strain and gained immunity,[20, 24] see Figures 2 and 3. Thus in Northwest Africa and in Cairo, Egypt, there is evidence of heavy exposure in early life to poliomyelitis virus. The infants there are very busy making antibodies during the earliest years of their life. Actually, by the time they have reached the age of five or six years they have reached immunological maturity as far as this

disease is concerned, thus eliminating the subsequent threat of adult poliomyelitis. This fact is also borne out by the extreme dearth of adult clinical cases in locally born residents of these areas.

Although the neutralizing antibody response recorded in Figure 2 is only that of one strain, i.e., Type 2, it appears that this antibody is a fairly good index of the immunity status of poliomyelitis in general.[24] The basis of this statement rests on the fact that in a number of widely separated areas, there is fairly good correlation, i.e., if the ages at which Type 2 *poliomyelitis neutralizing* antibodies have been acquired are compared with the ages at which *clinical poliomyelitis* (presumably due to any type) was acquired.

As to the speed with which these Type 2 poliomyelitis neutralizing antibodies are acquired, in contrast to the Cairo situation where it is about as fast as can be, one may choose a typical U.S.A. situation such as that in Charleston, West Virginia, where the curve in Figure 2 reflects a delayed rise in neutralizing antibodies which does not reach 90 per cent until the age of 30, indicating that adolescent and adult poliomyelitis is common, and immunological maturity has been postponed.

The above results record findings concerned with only one strain of the virus. With the introduction of tissue culture methods it is now possible to carry out neutralization studies with all three types. In general, the results of these triple surveys are the same as those done with Type 2 virus alone, indicating that in some populations infants are infected two, and sometimes three times in the early years of their life, so that antibodies to one, two, or three types are present in about 80 per cent of the population by the time they have reached five years (see Fig. 3).[22]

It has also been possible recently to carry out *complement fixation* tests on sera from a given population.[3] From analyses of the age specific rates at which these more transient antibodies are acquired or lost one can determine not only what the story has been with regard to poliomyelitis infections in the past but also those which have occurred recently, i.e., within three to five years. This is based on the assumption that complement fixing antibodies as measured in these studies do not remain elevated for longer than three to five years (see Figs. 2 and 3).

By including both neutralizing and complement fixation results in the juvenile population studies of Cairo, Northwest Africa and Charleston, West Virginia, one can compare the age incidence of recent versus old infection. Here we also have an opportunity to get some answers on the puzzling question as to the duration of immunity in poliomyelitis. Is it lasting and solid? Do type specific reinfections occur? Actually there appear to be at least two answers (see Fig. 2) to this, for one can find two different

types of complement fixation responses in different populations: one indicates (see left side Fig. 2) that in the Cairo population there is evidence that recent infections (whether new infections or re-infections) are all concentrated in childhood; whereas another in the city of Charleston, West Virginia, (see right side Fig. 2) indicates that these events are spread over a 30-year period, suggesting that immunity as acquired there was not so solid, and type specific reinfection may occur. This phenomenon calls for far more investigation, for it is clear that the work is in its infancy and that older qualitative measurements of age specific antibodies now deserve to be replaced by quantitative measurements.

In summary, therefore, it appears that both the disease, poliomyelitis and epidemiological thinking about it has undergone revision in the past generation. From an *endemic* disease it has become *epidemic* in many places, and indeed has become a common periodic scourge. From a disease originally regarded as being limited to infants, it is no longer confined to infancy. From a disease originally considered as mildly contagious or infectious, it is now regarded as highly infectious. From a disease in which it has been claimed that environmental sanitation played no part in its spread it has become one in which it is now clear that this factor must play some part.

A number of methods which utilize greatly improved techniques for the determination of antibodies against poliomyelitis virus have clarified some of the previously existing epidemiological puzzles by giving us some comprehension of the relative immunity status of various populations. In its new perspective, poliomyelitis now appears as a disease, spread not only through *direct contact* between individuals, but one in which contamination of an infected individual's *immediate environment* cannot be ignored. This is brought out by its varied behavior in different urban areas where sanitary arrangements vary. In communities where infant mortality is high, sanitation is primitive, and living conditions are crowded and poor, facilities for the spread of such a virus are better than elsewhere. Consequently, within such primitive communities, infants have the opportunity to come in contact with all three types of poliomyelitis virus early in life. Few of them reach the age of three or four years without having been infected at least with one strain, and most of them with two, although clinically, and officially the infection to a very large degree is inapparent. In any event, immunity is acquired rapidly and silently in such areas as far as local official morbidity statistics are concerned and no large group of susceptibles is built up. Within such communities the disease smoulders, and they are not liable to have epidemics of poliomyelitis. In countries where sanitary arrangements are no longer primitive, the risk of contact with the virus at an early age

is diminished. A large percentage of the juvenile population reaches the age of six, eight, or ten years without having acquired any infection or any immunity, and consequently such populations are ripe for epidemics, which sooner or later seem to appear. It might seem questionable whether this latter situation is desirable. This characteristic difference in the behavior of poliomyelitis in different places is the strongest evidence that the spread of this disease is influenced by environmental factors, a feature which for years has been controversial.*

Other and crucial questions here are whether poliomyelitis is really a much milder disease in infancy than it is later in life and whether the infantile ratio of apparent to inapparent infection is of the order of 1 : 200, in contrast to a childhood ratio of nearer 1 : 100. This may be based on the fact that the recent presence of residual maternal antibody, or as better stated, "resistance inherited from the mother," may equip the infant quite well to sustain a modified attack or attacks of poliomyelitis, if he is fortunate enough to be heavily exposed in infancy.

If there are any morals or lessons to be learned, it would appear that if one is to be concerned with the prevention of poliomyelitis, then the whole situation in this country is one which cries out especially for some means of artificial immunization as a far more hopeful approach than further attempts to rid the environment of poliomyelitis virus.

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* Space does not allow a discussion of the question as to whether the dominant effect of poor living conditions consists in crowding. By comparing different environmental conditions on another contact disease, namely, mumps, poor living conditions do not exert as much effect as in poliomyelitis.[18]

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BETA-TRACK AUTORADIOGRAPHIC STUDY OF TEETH WITH Ca^{45}*

Variations of calcium[45] taken up *in vitro* by the enamel and dentin of teeth have been reported recently. Dentin appears to adsorb more Ca^{45} than enamel, and carious dentin more than non-carious dentin. Underwood and Hodge[10] measured the Ca^{45} differences in solutions of powdered non-carious enamel and dentin. Minder and Gordonoff[7] have published autoradiographs with Ca^{45} of intact rat teeth, and Belanger[2] of dentin caries in a human tooth. Amprino and Camanni[1] described experiments with dog and human teeth. In the present study, autoradiographs were used to locate and measure the Ca^{45} taken up *in vitro* by sections of intact and carious human teeth.

METHOD

The method used was that of "track" autoradiography[3, 4, 5] which differs from "contrast" autoradiography as follows:

1. Track autoradiography involves microscopic study of the linear sequence of silver grains rendered developable in an emulsion by the passage of a charged (ionizing) nuclear particle. Contrast autoradiography consists of observation of paths of great numbers of nuclear particles in an emulsion appearing in the developed image as a dense mass of silver grains.

2. Readily detected tracks can be obtained with use of specifically designed nuclear particle emulsions and low energy beta-emitters, such as Ca^{45} (maximum energy 0.25 Mev), or alpha-emitters. Contrast autoradiography is possible with any radioactive isotope and a variety of emulsions.

3. Due to the nature of beta-particle traversal through an emulsion—that is, its ionization increases as its speed is decreased—tracks are obtained which at the starting point are far apart and at the terminal end are close together. Thus, the origin of the particle may be determined. With contrast autoradiography this is not possible.

4. With use of Ca^{45}, microscopic study of the location of individual tracks may be correlated with very small morphological structure. Contrast autoradiographs are a record of a large accumulation of tracks and consequently are limited to relation with large morphological structure.

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5. Microscopic study of tracks is possible with exposures much less than those necessary for measuring contrast of grain density.

6. By counting tracks the amount of radioactive tracer necessary for detection can be reduced.

If a tooth section is placed directly on the emulsion, or vice versa, the opaqueness of the calcified tissue limits the observation of the superimposed silver grains. Also, calcified tissue sections mounted permanently on emulsions are subject to harmful effects caused by the photographic processing solutions and the sections block the processing solutions from the underlying exposed part of the emulsion. These problems are avoided by the use of a method which separates the tissue and emulsion during processing and examination and yet maintains the correlation between small morphological structure and track data. The technique of Hoecker and Roofe[6] satisfies these requirements. Sections were mounted on a plastic cover slip which was bent back during processing, then returned to its original position.

Teeth used were obtained from patients of the Grace-New Haven Community Hospital Dental Clinic. After being fixed in 10% formalin, a random sample of these teeth was ground by hand to a thickness of 50-75μ. Five sections, approximately 5 mm. square, were placed in a Ca^{45} chloride solution of specific activity, 25 mc./gm.,* and a total calcium chloride concentration of 1×10^{-2} M.

Sections were mounted with Canada balsam or synthetic resin on one end of a 60 mm. flexible plastic cover slip. In total darkness, the other end of the cover slip was glued to a nuclear track plate (Kodak NTB, 10μ). Sections and emulsion were allowed to touch with light pressure for exposure times varying from 15 minutes to 24 hours. After exposure the mounted section end of the cover slip was bent and clamped away from the exposed end of the track plate in order to avoid contact of the tissue sections and the photographic processing solutions. The plates were developed for four minutes in Kodak D-19, stop-washed three minutes in water, fixed in Kodak acid fixer for 20 minutes, washed in running water for 30 minutes, and dried in air.

Sections were examined with a binocular microscope, and areas of dentin which were to be correlated with track counts were selected at random. Areas of enamel were selected which had an approximately equal width. Without moving the microscope stage, the cover slip with the opaque sections was bent out of the way so that the corresponding area of the exposed track plate could be examined. Exposure and examination were performed at room temperature to avoid appreciable dimensional change of the plastic cover slip. Shrinkage of the emulsion during processing occurs almost wholly in a direction perpendicular to the glass backing[8]; thus, only the total length and not the location of the track is affected.

Track counting was performed at 1350x (oil immersion). A reticle ruled with a square net was inserted into the microscope eyepiece and calibrated with a stage micrometer. At the magnification used, a 75μ square area was delimited in the field of vision. Tracks were counted in a zone across the emulsion, corresponding to a similar zone, 75μ wide, across the tooth structure. A track was defined arbitrarily as seven or more silver grains in a row, and only tracks originating in the delimited square area

* Measurements of radioactivity were performed through the courtesy of Dr. E. R. Gabrieli and Dr. J. H. Heller, Section of Medical Physics, Departments of Physiology and Internal Medicine, Yale University School of Medicine.

were counted. Few tracks were projected entirely in the same focal plane (horizontally); thus, adjustment of the fine focus control was necessary to follow the vertical component of each track in the emulsion.

Prior to moving the field of vision, a recognizable group of silver grains was noted on one edge of the square reticulum, then these silver grains were moved to the

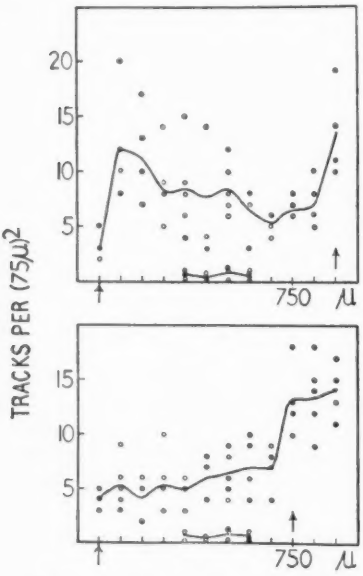

FIG. 1. Track counts on autoradiographs of carious (upper graph) and non-carious (lower graph) human enamel.

Ordinate: Number of tracks per unit area.
Abscissa: Distance along a zone counted across the emulsion, corresponding to a similar zone across the enamel.
Left arrow: Outer edge of enamel.
Right arrow: Beginning of dentin.
Circles: Values for one or more sections (some values were similar).
Curves: Mean values for five different teeth. Short curves indicate background.

Sections were immersed in a Ca^{45} solution for one hour. Track plates were exposed one hour.

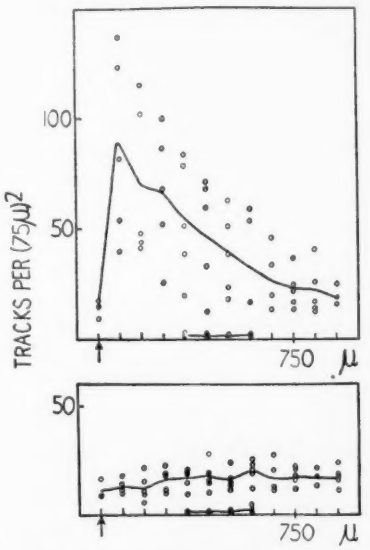

FIG. 2. Track counts on autoradiographs of carious (upper graph) and non-carious (lower graph) human dentin.

Ordinate: Number of tracks per unit area.
Abscissa: Distance along a zone counted across the emulsion, corresponding to a similar zone across the dentin.
Arrow: Outer edge of dentin.
Circles: Values for one or more sections (some values were similar).
Curves: Mean values for five different teeth selected at random. Short curves indicate background.

Sections were immersed in a Ca^{45} solution for one hour. Track plates were exposed one hour.

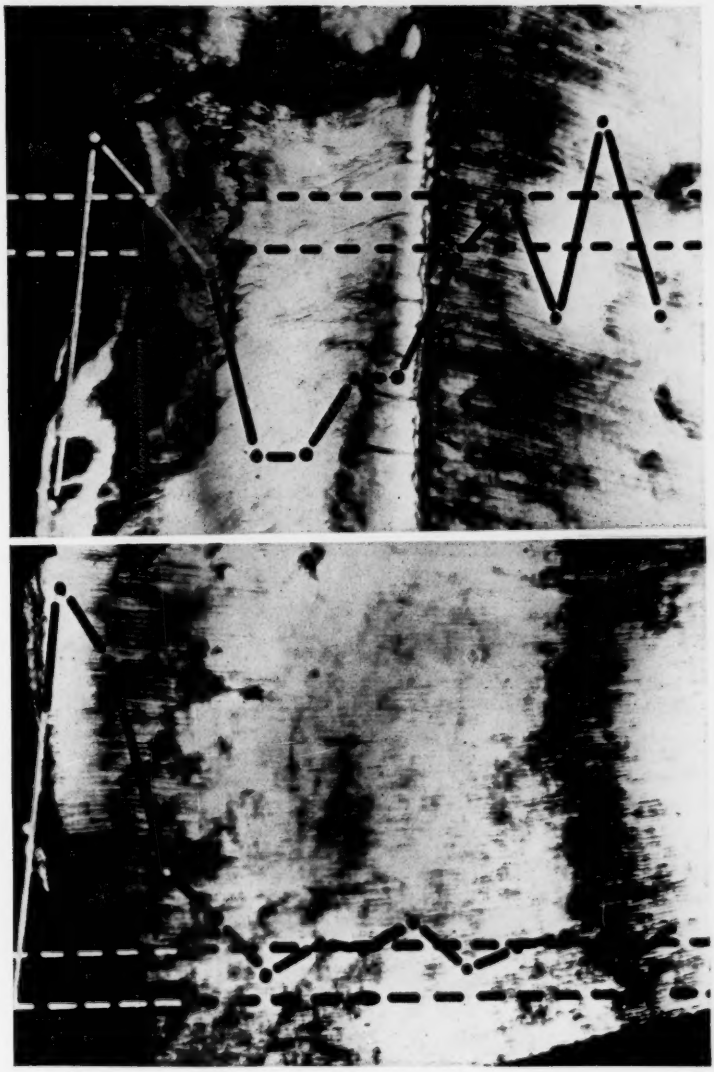

FIG. 3. Photograph of beginning caries in enamel. Superimposed curve shows distribution of tracks in corresponding autoradiograph. Section similar to those in Figure 1.
Dotted lines: Boundaries of the zone in which track counting was performed.
Abscissa: Lower edge of photograph.
Magnification: 110x.
Photographed with plane polarized light.

FIG. 4. Photograph of caries in dentin. Superimposed curve shows distribution of tracks in corresponding autoradiograph. Section similar to those in Figure 2.
Dotted lines: Boundaries of the zone in which track counting was performed.
Abscissa: Lower edge of photograph.
Magnification: 110x.
Photographed with plane polarized light.

opposite edge of the reticulum by slight rotation of the micrometer stage screw. Sections were usually oriented with the dentin-enamel junction parallel to one edge of the cover slip, which in turn was parallel to a direction of movement of the microscope stage. The square counting field did not extend to the edges of the field of vision; hence, errors due to parallax were not appreciable.

RESULTS

The results were plotted as curves with abscissa: the unit of distance along the counting zone, in this instance, 75μ; and ordinate: the number of tracks per unit area counted, the unit area being $5625\mu^2$. Some curves were superimposed on photographs of the corresponding sections in order to demonstrate which areas in the tooth section coincided with zones of high track counts. The distances from the edges of the tooth section to the track counting zone were measured; and all track photographs were accompanied by a photograph of a stage micrometer at the same magnification. A track curve transfer to a photograph of the corresponding section was then possible with an error of less than 5μ.

Figure 1 shows tracks counts through enamel. The number of tracks in areas corresponding to "beginning" caries may be compared to areas of no caries in the same teeth. The Ca^{45} uptake by carious enamel is increased when compared to non-carious enamel. Also, one may note an increase in the Ca^{45} uptake by dentin as compared to enamel.

Figure 2 indicates a great difference of Ca^{45} uptake between carious and non-carious dentin. The spread of the carious dentin values is probably due to differences in degree and extent of the caries attack. These data are from sections immersed in a Ca^{45} solution for the same period of time as those in Figure 1. Exposure times for the track plates also were the same.

Figures 3 and 4 show track count curves drawn on photographs. Areas of increased Ca^{45} uptake, as measured by track counts, appear to correlate with the caries, as observed by plane polarized light. In unstained teeth sections, the definition of caries may be observed more readily with polarized than non-polarized light because of a variation in sign of the usual birefringence.[9]

By means of track autoradiography, small differences in the distribution of Ca^{45} may be related with very small morphological structure. Detection of minute amounts of Ca^{45} is possible, and hence, the amount of Ca^{45} used can be reduced considerably. Opportunity is offered to measure the degree and extent of dental caries as compared to areas of no caries. Further investigations are in progress.

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AN APPLICATION OF THE PAPER ELECTROPHORESIS TECHNIQUE TO SERUM CHANGES IN ARTERIOSCLEROSIS*

INTRODUCTION

Until recently, blood lipid changes seen in arteriosclerosis were followed mainly by chemical means. With increasing awareness, however, of the extreme lability of the compounds and complexes which make up serum or plasma, physical methods have been used extensively.

Cohn and Edsall's[2,7] system for plasma fractionation has made possible detailed studies on blood lipids and lipoprotein complexes. The work carried out by Gurd and Oncley[18] led to a better understanding of the composition of these fractions. The application of the ultracentrifugation technique by Gofman[9] revealed the important fact that lipoproteins of certain flotation rates (especially S_f 10-20) are present in blood and are associated with symptomatic arteriosclerosis; these unusual complexes were also found in rabbits with the cholesterol-induced form of the disease.

Phase boundary electrophoresis has not been widely applied to the study of arteriosclerosis, although a modification of this technique, paper strip electrophoresis, is receiving considerable attention as an analytical tool.

Initial work with this method was done mainly on proteins, and numerous variations and modifications have been developed using a wide variety of stains in an effort to improve quantitative measurement of the fractions.[4, 5, 10, 13, 28] In 1952 two studies appeared where the paper electrophoresis method was adapted for the study of lipoproteins and cholesterol.[12, 19]

The work discussed here was undertaken with a two-fold purpose in mind: (a) to evaluate the general method as a whole; and (b) to apply this technique to the study of sera from arteriosclerotic patients in an attempt to shed some further light on the pattern of blood changes occurring in this disease.

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* The material here presented is taken from the thesis of Nicholas A. Halasz submitted to the Faculty of the School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine in 1954.

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EQUIPMENT

Several types of electrophoretic chambers were employed during the course of the experiments.

(a) Durrum's "chalet-type" chamber.[5]

(b) A horizontal chamber, 16½" x 13" x 3½" made of ⅛" lucite. Two lucite buffer plates were inserted into each electrode chamber to reduce ionization. Electrodes were made of carbon plates measuring 12" x 2" x ⅜". In the horizontal chamber, the paper strips were kept from sagging by inserting each end of the paper through a slotted lucite rod which was then turned until the paper was put under tension. The two ends of the paper were then dipped into their respective electrode chambers.

(c) A vertical chamber 14" x 11" x 8", made of ⅛" lucite, with removable lid and built-in thermometer proved to be most satisfactory. Electrodes were of carbon, 12" x 1" x ¼". Paper strips were draped across a 1" lucite plate, 10" above the level of the buffer. Two vertical buffer plates were again set into the electrode chambers. The two electrode-cells were connected in this, as well as the other chambers used, by means of plastic tubing emerging under the level of the buffer in order to equalize the fluid levels prior to migration and thus prevent syphoning through the paper strips. During the electrophoretic separation, these tubes are clamped tightly to insure insulation of the two cells.

Filter paper: At first large sheets of Whatman No. 1 paper were cut into 1½ inch strips of appropriate length for the chamber used, but later, paper of the above specifications became available in 500 foot rolls and was used because of greater convenience.

Power supply: A constant voltage power supply was used which had a maximum output of 100 milliamperes and 2,000 volts.

Capillary pipettes: Microanalytical type delivering down to 0.01 cc.

Reagents: Barbiturate buffer[16]:

Sodium barbiturate	72.16	gm.
Barbituric acid	12.89	gm.
Water q.s.	3,500	cc.

Five hundred cc. of this stock solution was diluted to 1,200 cc. with water, yielding a buffer of μ: 0.1 and pH: 8.6.

Flavianic acid[21]: 1% aqueous solution.

Amido schwarz[10]: 1 gm. in 900 cc. methanol and 100 cc. glacial acetic acid.

Washing solutions for Amido schwarz:

1. 900 cc. methanol and 100 cc. glacial acetic acid.
2. 0.5% glacial acetic acid.

Sudan III: saturated solution in 50% ethanol.

Oil red O[6]: saturated solution in 50% ethanol.

Antimony pentachloride[15, 22]: 20% solution in chloroform.

Colorimeter. The Evelyn colorimeter was used after modification as follows. The test tube holder was removed and a ⅛-inch bakelite strip cut to fit closely was inserted in its place. This piece projected about 3 inches above the top of the instrument when fully inserted. A window, ¼ of an inch wide, was cut out of the bakelite. This was placed so as to lie opposite the center of the photocell when the strip was inserted.

A piece of $\frac{1}{32}$-inch celluloid was then cut to fit next to the bakelite slab on its side next to the light source. A guide rail was inserted to insure that the assembly was at a 90° angle to the path of light.

PROCEDURE

Specimens: All blood samples were collected after an overnight fast, permitted to clot in a sterile tube, and the serum then separated by centrifugation. Sera were kept at 4° C. and analyzed within 48 hours to minimize alterations of the lipoprotein complexes.

Electrophoretic separation: A strip of filter paper of appropriate length was marked with a line to denote the origin of migration, wetted thoroughly in the buffer solution, and blotted briefly with another sheet of filter paper. The area of the point of the migration was then blotted further and 0.015 to 0.03 cc. of serum was applied in a streak over the start line with a capillary pipette. The paper was then placed across the supporting stand or plate of the chamber, using cover glass forceps, and its ends were allowed to dip about ½ of an inch into the buffer solution of the respective cells. The entire chamber was covered, sealed with vaseline, and the connecting tubing clamped off. A constant potential varying between 250 to 400 volts was then applied across the strips for periods varying from 4 to 16 hours.

Drying: Strips were usually processed in groups of three, having been stained for protein, lipid, and cholesterol respectively. After the current was turned off, the strips were removed at once from the chamber and clipped to a glass rod by their ends. To avoid tearing the paper and to prevent artefact stained spots, care must be taken not to touch the strips over the stained area with the fingers or other objects. The strips were then dried in a hot-air oven at 80° C. for 15 minutes. This serves not only to dry the paper but to "fiix" the proteins as well.

Protein staining: The dry strip was immersed in 0.1% Amido schwarz solution for 10 minutes. A constant washing motion of the dye solution was insured by placing the staining dish on a slowly moving electric shaker. Three successive washings in baths of the methanol-acetic acid mixture followed, again using the electric shaker for five minutes each time. Final rinsing was done in the 0.5% acetic acid solution. The strip was then placed on a glass plate and dried in hot air.

Lipid staining: The strip was soaked for 24 hours in the oil red 0 solution, and then thoroughly washed in running tap water. Drying was done as for the protein-stained strips.

Cholesterol staining: The dry strip was suspended in a hood and a weight attached to its bottom to prevent it from flapping. It was then sprayed with antimony pentachloride solution, until the surface was uniformly wetted. After three minutes it was rinsed in running water, and dried between filter papers compressed by heavy plate-glass to prevent warping. The paper comes out stiff but does not become brittle before 48 hours have passed.

Colorimetric reading: The stained and dried strips were immersed in mineral oil and drained of any excess by blotting. They were then individually inserted into the colorimeter between the bakelite and celluloid strips, at first in such a fashion that the most heavily stained area faced the window of the photocell. The galvanometer of the

colorimeter was then set at 0 or 100 depending on whether transmission or density was plotted on the scale. The strip was then reinserted so that the upper edge of the stained area faced opposite the window. A galvanometric reading was taken, the strip was moved up by ¼ inch (i.e., the width of the window), another reading taken, and so on until the light transmission (or density) of the entire stained area had been compared. Protein lipid and cholesterol patterns were read in the Evelyn colorimeter using filters 540, 420, and 490 respectively.

Plotting of results: The photoelectric readings obtained were plotted on graph paper against the distances traveled by the fractions from the initial point of the migration. All three patterns (protein, lipid, and cholesterol) were superimposed on the same sheet. If more than relative quantitation was desired, another set of strips was processed with approximately the expected amounts of the analyzed substances to standardize the photoelectric readings. The areas covered by the peaks in the pattern were then measured using a planimeter.

Chemical determinations: Cholesterol analyses were done by the method of Bloor *et al.*[1] for total cholesterol. Lipid phosphorus was measured by the technique described by Fiske and Subbarow.[8] Albumin-globulin ratios were determined by the ammonium sulfate precipitation method using the micro-Kjeldahl procedure.

EXPERIMENTAL RESULTS

As a preliminary to the study, a survey of some of the biological stains applicable to paper electrophoretic method was undertaken. Since the stain on the strips was to be measured directly on the paper without elution, a special effort was made to select a dye for each substance under analysis which had a high color intensity as well as a low affinity for the paper itself. This minimizes "background staining" (i.e., the adsorption of stain in areas where none of the substance in question was deposited):

Proteins: Twenty stains were studied using serum as well as pure albumin and globulin fractions. Various solvents for the stain and rinses to remove unadsorbed dye were employed. These results are summarized in Table 1. It was found in general that a solvent for the stain which will precipitate or denature protein is preferred. It will not only prevent losses of protein into the staining solution by elution, but also fixes it to the paper. This affords protection against diffusion of poorly adsorbed fractions on the strip which results in unclear patterns.

Lipids: Serum and various lipid complexes were used to test eight biological fat stains. The results are charted in Table 2. Alcohol-water mixtures had to be used as solvents for most of the dyes, but there was no significant loss of lipid material from the paper into the stain. The major difficulty encountered was the low specificity of the so-called "fat-stains" for lipid material in the serum and their relatively strong adsorption on the paper not containing fat. Oil red 0 was found to be the most satisfactory

TABLE 1. PROTEIN STAINS

Stain	*Solvent*	*Rinse*	*Sensitivity*	*Background staining*	*Overstaining*
Acid Fuchsin	H_2O	H_2O	poor	3+	
Alizarin	EtOH	EtOH	poor	2+	
		Acetone	poor	2+	
Amido schwarz	10% HAc in methanol	same	excellent	1+	globulins 10%
Brilliant green	H_2O	H_2O	poor	2+	
	EtOH	Acetone	fair	4+	
Bromcresol blue	H_2O	H_2O	poor	3+	
		EtOH	poor	3+	
Bromcresol green	H_2O	H_2O	poor	1+	
	EtOH	EtOH	poor	2+	
Bromcresol purple	H_2O	H_2O	fair	2+	
	EtOH	EtOH	good	3+	
Bromphenol blue	HAc, $HgCl_2$	HAc	good*	trace	albumin 25%
Crystal violet	H_2O	H_2O	poor	3+	
	EtOH	EtOH	poor	2+	
Flavianic acid	H_2O	H_2O	excellent	0	globulins 20%
	EtOH	EtOH	fair	0	(esp. γ-fraction)
Light green SF	H_2O	H_2O	fair	2+	
	methanol	methanol	good	2+	
Methyl orange	H_2O	H_2O	poor	3+	
Methyl red	EtOH	EtOH	poor	1+	
Nigrosine	EtOH	Acetone	fair	2+	
Orange G	EtOH	EtOH	poor	1+	
	acetone	Acetone	fair	3+	
Phloxin B	EtOH	EtOH	fair	1+	
		EtOH + acetone	good	2+	
Safranin	H_2O	H_2O	poor	1+	
	EtOH	EtOH	fair	3+	
Wright's stain	EtOH	EtOH	fair	trace	
		Acetone	fair	trace	
		$CHCl_3$	good	2+	
Orange G, Phloxin B	EtOH	EtOH	good	3+	
Wright's, Phloxin B	EtOH	Acetone	good	1+	
		EtOH	excellent	trace	**

* Stain gradually fades on exposure to light.

** Stains albumin very unreliably and with great variations in color intensity.

stain available and was more satisfactory than Sudan III, used extensively in previous studies.

Cholesterol: Using serum and cholesterol, the various available color reactions for this substance were studied. Although Rosenberg in 1952[19] reported using the Schultz modification of the Liebermann-Burchard reaction with good results, all the techniques employing concentrated H_2SO_4 had to be abandoned because the acid absorbed moisture from the air and destroyed the paper. Nyc's[17] method using zinc chloride was not found to be sensitive enough for the small amounts of cholesterol found in serum. The best reagent was antimony pentachloride, as first described by Steinle and

TABLE 2. LIPID STAINS*

Stain	*Solvent*	*Sensitivity*	*Background staining*
Sudan II	50% ethanol	fair	3+
Sudan III	50% ethanol	good	1+
Sudan IV	50% ethanol	fair	2+
Coccinel red	Isopropyl alc.	poor	trace
Oil blue N	Isopropyl alc.	poor	3+
Rhodamine	50% ethanol	fair	2+
Thioflavin S	50% ethanol	fair	1+
Oil red O	50% ethanol	excellent	1+

* The rinse for the stains in all these experiments was distilled water.

Kahlenberg in 1926[22] and more recently by McMahon.[15] This reagent gives a brownish-yellow color with cholesterol and does not decompose the paper.

Lipid phosphorus and carotenoids: None of the available chemical methods were found to be sensitive enough to stain these substances on the strip under the conditions of these experiments.

Analysis of normal sera: Serum from 27 individuals was studied. The group contained 6 females and 21 males and ranged from 5 to 27 years in age, the mean being 21.6. Care was taken not to include in this group any individual with a history of vascular, renal, or hepatic disease, and also to exclude those with acute or chronic metabolic or debilitating diseases. Cholesterol (total) and phospholipid determinations were performed on these sera with the resuts all falling within the prescribed normal limits.

Figure 1 shows a composite diagram of the protein, lipid, and cholesterol patterns obtained using these normal sera. The arrow points to the initial point of the migration. The gross distribution of the lipid and cholesterol in relation to the plasma protein was as expected, with peaks in the beta

globulin and albumin-alpha$_1$ globulin areas. However, of interest is the dissociation and slight displacement of the lipid peaks ahead of the cholesterol, which was a consistent and reproducible finding in these studies. The accuracy of the protein quantitation as measured by A/G ratios is satisfactory, as seen in Table 3. There was no adequate way available to check the accuracy of the fractional cholesterol and lipid representations

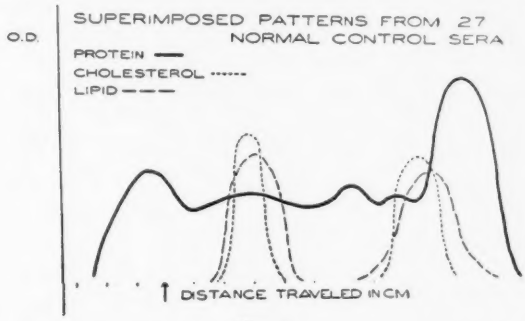

FIG. 1.

on the paper strips; therefore, these results are of a relative nature from one serum sample to another. The configuration of the tracings was the same in all these analyses of normal sera. Differences in serum concentrations caused quantitative changes in the patterns without altering their typical configuration or the distribution of the various fractions.

Reproducibility of the various patterns was found to be satisfactory, as can be seen from the following results of triplicate analyses performed on 15 sera:

Substance tested for	*Variation in O.D.**
Protein	± 2.7%
Lipid	± 4.3%
Cholesterol	± 5.8%

(*O.D. = 2 –log G, where G is the galvanometric reading.)

The usual albumin-globulin ratio determinations were performed on 11 of the control sera. The results of these tests are compared with the electrophoretically obtained ratios in Table 3. The albumin/globulin ratios obtained by paper electrophoresis are about 20% higher on the average than

those measured by the chemical method. This might be expected on the basis of the incomplete separation obtained by the later method. It is clear that the values obtained by the newer method should be checked against a chemical standard, but the relative quantitation it gives is adequate and can be relied on for comparative evaluations.

The plane in which the strip lies during migration appears to have little effect on the rate of travel of the various substances. However, it does have a definite influence on the clarity of the patterns obtained. Thus, downward migration with the strips in a near-vertical or vertical position gives a

TABLE 3. COMPARISON OF KJELDAHL AND PAPER ELECTROPHORESIS A/G RATIOS

A/G ratio by:	*Kjeldahl*	*Paper electrophoresis*
1	1.3	1.6
2	1.5	1.8
3	1.2	1.5
4	1.3	1.5
5	1.2	1.4
6	1.5	1.8
7	1.3	1.5
8	1.4	1.7
9	1.6	1.9
10	1.4	1.7
11	1.5	1.9

clearer definition of the various fractions than does the horizontal migration technique. The principal advantage of the horizontal migration procedure is an almost complete elimination of the back-migration of the gamma globulin fraction (i.e., travel in the direction opposite that of the other protein fractions). At the present time there is no adequate explanation for this phenomenon, but it probably is related to an important physical phenomenon observed by Conn[8] in vertical chambers. He found that in a solvent-saturated atmosphere the rate of evaporation is greatest at the apex of the tented vertical strip. This is supported by finding the concentration of the buffer also to be highest at this point. A corollary of the above is the postulation of a steady, slow, upward flow from both ends of the paper—dipping into the buffer—towards the apex of the strip. Therefore, when migration takes place in a downward direction, the traveling substances have to "buck the stream" and this is probably the chief factor responsible for a more clearcut resolution obtained by the vertical method.

The back migration is probably due to the low migration speed of the fraction involved, and a delicate balance between the opposed electric force and "drag" of the flowing buffer.

The extreme sensitivity of the strips has to be noted. Not until the strip is dried after the staining procedure is it safe to touch it. Touching before migration will interfere with the even wetting of the strip by the buffer. After separation, the wet strip is at its tenderest. It tears, because it has been soaking for several hours in an aqueous medium, and the lightest touch with even a chemically clean object will result in a heavy deposit of stain because of the alteration in the surface. Cholesterol strips require special care, especially in summer, because of contamination by perspiration. In general, strips should be handled by their ends which do not serve as matrix for further migration and whenever possible with coverglass forceps or, if necessary, with dry clean hands.

Pathological sera: Thirty-one patients, 20 males and 11 females, were studied on the wards of the Grace-New Haven Community Hospital. Average age of the group was 62.4, ranging from 46 to 93 years. Since no absolute criteria are available for the classification or estimation of the degree and extent of vascular involvement in this disease, the following arbitrary groups were established.

Group I. Cases with proven myocardial infarctions. Seventeen patients, 12 males, 5 females.

Group II. Cases with cerebral vascular accidents without evidence of specific local pathology. Only patients with a systolic blood pressure below 180 mm. Hg and a diastolic below 110 mm. Hg were included in this group, with Grade II or less hypertensive retinal pathology. Six patients, 3 males, 3 females.

Group III. Normotensive diabetics with peripheral vascular insufficiency, necessitating amputation of more than fingers or toes. Five patients, 3 females, 2 males.

Group IV. Non-diabetic patients with peripheral vascular disease requiring amputation, where the only pathology found was arteriosclerosis. Three patients, all males.

The patterns obtained in this group were of two types. Twenty patients showed a normal distribution of the various fractions, with occasional aberrations within the normal pattern. Eleven patients, however, had a qualitatively different fractional pattern, of a type not hitherto seen or described ("Type B").

Pattern A: This is the picture comparable to that obtained in the control group. Eight of the 20 patients (40%) in this group showed an elevated

serum cholesterol and four (20%), an elevated serum phospholipid by chemical methods. These abnormalities were grossly identifiable on the electrophoretic patterns, when the cholesterol was elevated 10% or more and the phospholipid 20% or more. However, the increase in the total serum concentrations resulted only in a quantitative change in the pattern. The excess was added onto the normal peaks, their configuration and relative location remaining unchanged.

Pattern B: Eleven patients demonstrated this pattern (see Fig. 2). Seven of these (63.6%) had elevated serum cholesterol levels and three

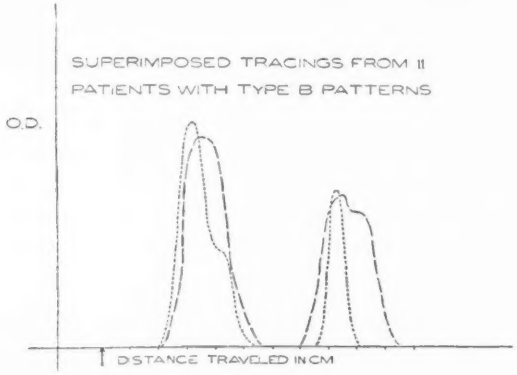

FIG. 2.

(27.3%), elevated phospholipids. This pattern showed qualitative as well as quantitative deviation from the previously established normal pattern (type A, above). The characteristic changes consisted in an extension of the cholesterol peak associated with the beta globulin, and a similar extension of the lipid fraction migrating in the albumin-alpha$_1$ globulin region. Neither of these extensions showed a definite peak separate from the main ("normal") fraction, instead the latter extended in a plateau rather than taking its normal parabolic configuration. In both cases the additional fraction migrated at a somewhat faster rate than the main peak it was associated with.

The "abnormal" type B pattern was reproducible within the same tolerances as the normal A patterns. Samples drawn repeatedly from four patients who on previous examinations had a type B pattern revealed its persistence. In four cases a second sample was drawn 3, 4, 7, and 13 months after the initial examinations, and in one case two follow-up samples 6 and

12 months after the first one. In all these cases the patterns remained unchanged although the hypercholesterolemia in the last case (#17) decreased to 237 mg. % after 6 months and to 192 mg. % after one year on a modified rice diet.

TABLE 4. PATHOLOGICAL SERA

Pt	*Sex*	*Group*	*Cholesterol*	*Phospholipid*	*C/P ratio*	*Type of tracing*
1	F	III	187	210	.89	A
2	M	I	230	196	1.17	B
3	M	IV	285	263	1.08	A
4	M	I	161	143	1.12	B
5	F	II	307	164	1.87	B
6	M	I	182	201	.90	A
7	M	I	269	191	1.41	A
8	F	III	180	313	.58	B
9	M	I	276	294	.94	A
10	M	II	152	192	.79	A
11	F	I	167	184	.90	A
12	M	IV	184	177	1.04	B
13	M	I	257	201	1.28	A
14	F	I	283	180	1.57	B
15	F	III	171	155	1.10	A
16	M	I	184	163	1.13	A
17	M	I	290	188	1.54	B
18	F	II	168	157	1.07	A
19	M	IV	159	181	.88	A
20	F	I	175	276	.63	A
21	M	II	153	300	.51	B
22	M	I	309	158	1.96	B
23	M	II	138	182	.76	A
24	F	I	321	162	1.98	A
25	F	I	291	176	1.65	B
26	M	III	300	282	1.06	B
27	M	III	279	164	1.70	A
28	M	I	185	293	.63	A
29	M	I	153	180	.85	A
30	F	II	283	165	1.71	A
31	M	I	311	204	1.52	A

Average cholesterol concentration in pathological group: 225 mg./100 cc.
Average phospholipid concentration in pathological group: 203 mg./100 cc.
Cholesterol/phospholipid ratio:
group with type A tracing: 1.11
group with type B tracing: 1.28
average: 1.17

The clinical estimate of the severity of arteriosclerosis did not seem to be related in any way to the type of pattern obtained, neither, as can be seen in Tables 4 and 5, did the type of arteriosclerotic involvement. The type B

TABLE 5. PATHOLOGICAL SERA

		Gr. I	*Gr. II*	*Gr. III*	*Gr. IV*	*Total*
No. of patients with:		17	6	5	3	31
type A pattern	No.	11	4	3	2	20
	%	64.7	66.7	60.7	66.7	64.5
type B pattern	No.	6	2	2	1	11
	%	35.3	33.3	40.0	33.3	35.5
Normal cholesterol and phospholipid conc. with:						
type A pattern	No.	4	3	2	1	10
	%*	36.4	75.0	66.7	50.0	83.3†
type B pattern	No.	1	0	0	1	2
	%	16.7	0	0	100.0	16.7
Elevated cholesterol concentration with:						
type A pattern	No.	5	1	1	1	8
	%	45.5	25.0	33.3	50.0	53.3
type B pattern	No.	5	1	1	0	7
	%	83.3	50.0	50.0	0	46.7
Elevated phospholipid concentration with:						
type A pattern	No.	3	0	0	1	4
	%	27.3	0	0	1	4
type B pattern	No.	0	1	2	0	3
	%	0	50.0	100.0	0	42.9

* Expressed as % of patients in the group demonstrating this pattern (vertical grouping according to pathology—see page 13).

† Expressed as % of patients with comparable serum cholesterol and phospholipid concentrations demonstrating this pattern.

pattern, however, was more frequently seen in patients with elevated serum cholesterol or phospholipid concentrations than in those with normal values.

Distribution of the abnormal pattern between males and females was the same as that for the entire group. Fourteen patients had been receiving digitalis at the time the specimens were drawn with no demonstrable effect on the pattern.

In an attempt to eliminate the possible rôle of ageing without symptomatic arteriosclerosis, sera from eight well individuals with an average age of 62.3 (range: 54 to 71 years) were analyzed. Average serum cholesterol and phospholipid concentrations were slightly higher than in the young controls (12% and 9% respectively) but in none of these sera was the type B pattern found.

DISCUSSION

Studies on the normal control sera yielded results agreeing with the observations of Kunkel and Slater[12] and Rosenberg[19] excepting one finding. In none of our tracings were the lipid and cholesterol peaks found to coincide exactly. The lipid fractions consistently migrated somewhat ahead of the cholesterol, as seen in Figure 1, moving at a higher speed. This phenomenon may be a reflection of the heterogeneity of lipoproteins (containing cholesterol, fatty acids, and phospholipids), the differential migration rates pointing to separation within the larger subdivisions. Further electrophoretic analysis of the fractions in question might help to elucidate the problem.

Chemical determinations on the pathological sera were at some variance with those described by Russ *et al.*[20] on 14 arteriosclerotic patients. Using the method of Lever, Gurd *et al.*[14] to determine cholesterol levels, they observed a mean value of 273 mg. per 100 cc. The average in the present study was lower, only 225 mg. per 100 cc. However, a proportional difference was found in the values obtained on "normal" sera, therefore the variation in methodology may be assumed to account for the differences observed. The incidence of abnormally high cholesterol concentrations is quite similar, Barr having found 7/14 or 50%, while the present group demonstrated an incidence of 15/31 or 48.4%.

The results of the electrophoretic studies on the pathological group, as seen in Table 5, are significant, but not conclusive as such. Only 35.5% of this group showed the abnormal, type B pattern. However, of those with normal cholesterol and phospholipid levels, only 16.7% had this pattern while patients with elevated cholesterol and lipid phosphorus concentrations demonstrated it in 46.7% and 42.9%, respectively.

The presence of a normal, type A pattern in a larger number (64.5%) of patients with symptomatic arteriosclerosis well demonstrates that the abnormal, type B, tracing is not a *sine qua non* of the disease. Instead, it seems to be more of a reflection of disturbed cholesterol-phospholipid metabolism which has long been assumed to be involved in the pathogenesis

of the illness. Even in the group with abnormally elevated blood levels of these substances the incidence of the type B pattern is below half. Therefore, the presence of elevated cholesterol or phospholipid concentrations does not produce without fail the abnormal pattern, although there is a definite association since only 16.7% of those with normal blood levels demonstrated it. None of the normal controls having shown the type B pattern, one has to conclude that it must be related either to age or to the disease—the only differences between the two groups. Its absence from the "old controls" group finally links the type B pattern with the disease.

As to the genesis of the abnormal pattern the following hypothesis might be advanced. An abnormality inherent in the serum lipids may result in the distortion of the normal tracing, by the production of new fractions, migrating at speeds different from that of the normal constituents. This interpretation gains support from the work of Gofman *et al.*[8] who did find lipoprotein complexes of a different nature in atherosclerotics. Also, the alterations characterizing the type B pattern were always found to occur together. In no tracing was an abnormal cholesterol or phospholipid pattern found, unaccompanied by the typical change in the other. It is remarkable not only how reproducible the pattern was once it had occurred, but that it always involved both components. This fact suggests an interdependent change, involving both components and being reflected by the appearance of new fractions in the electrophoretic pattern. Despite the fact that no alterations were found to occur in the protein pattern, some change in the globulins might be postulated occurring with the disease. This may prevent the normal transport of the lipid material in the blood, leading to an "overflow" into globulin fractions not usually involved in this transport. Russ *et al.*[30] have demonstrated a decrease in the alpha globulin and albumin fractions in the disease. Similarly, in certain diseases of probably different etiology but associated with marked hyperlipemia, such as nephrosis and familial hypercholesterolemia, both qualitative and quantitative changes have been found to occur in the proteins, and quite possibly our present methods are inadequate to confirm minor changes which may be present in atherosclerosis.

Further experimental work should make some clarification possible. An attempt to produce the type B pattern experimentally should be of great value.

Hepatic function is another facet which may bear investigation if proteins should be involved in the pathogenesis of the disease. In this study no correlation was found between abnormal liver function tests and the presence

of the type B pattern, but only eight patients had tests performed and of the two with a significant abnormality, one had a normal, the other a type B pattern.

SUMMARY

1. A survey of the stains used in paper strip electrophoresis was carried out to re-evaluate these substances. Amido schwarz, oil red 0, and antimony pentachloride were found to be most suitable for protein, lipid, and cholesterol staining, respectively.

2. Twenty-seven normal sera were analyzed chemically for cholesterol and phospholipid, then studied electrophoretically, using the above stains and a direct reading method for the quantitation of the stain adsorbed on the strip. These results correlated well with those of other workers, except for the finding of some further separation within certain fractions.

3. Sera from 31 arteriosclerotic patients were studied by the above methods. One-third of this group demonstrated a basically different pattern configuration, reflecting changes in both the cholesterol and lipid fractions. Since these changes were more frequently seen in those cases with elevated cholesterol and lipid phosphorus concentration and since both of these fractions were always involved in the formation of the abnormal pattern, an interdependent change must be postulated involving excesses of both cholesterol and lipids as well as a possible abnormality in the serum.

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THE SYNTHESIS OF OROTIC ACID-$C^{14}O_2H$

Orotic acid-$C^{14}O_2H$, which was required for biological studies being pursued in this laboratory, has been synthesized by the method of Nyc and Mitchell,[3] starting with DL-aspartic acid-α-$C^{14}O_2H$. The latter was prepared from ethyl malonate-$C^{14}O_2C_2H_5$, as described by Galat.[2]

This orotic acid probably contains the isotopic carbon exclusively in the carboxyl group since it evolves carbon dioxide containing most of the original radioactivity when heated to 345° and the uracil which remains is not radioactive; this finding lends strong support to the mechanism proposed by Nyc and Mitchell (*loc. cit.*) for the alkaline rearrangement of 5-(carboxymethylidene)-hydantoin to orotic acid.

METHOD

ETHYL MALONATE-$C^{14}O_2C_2H_5$

Barium carbonate-C^{14} (150 mg., 5 mc., Oak Ridge) was converted by the method of McCarter[4] into sodium cyanide-C^{14}, this was then mixed with a solution of sodium cyanide-C^{12} (3.00 g.) in water (8 ml.). The resulting solution was used for the preparation of malonic acid, as described by Weiner.[7] The reaction was conducted in a centrifuge tube (50 ml.) and the crude calcium malonate obtained was separated by centrifugation and washed twice by stirring with ice-water (10 ml.), centrifuging after each addition. The process was repeated three times with acetone, and the product, after being dried in air, was converted into malonic acid in the usual way (Weiner, *loc. cit.*). This crude malonic acid (4.26 g.) was treated with absolute ethanol (50 ml.) and concentrated sulfuric acid (0.5 ml.). The mixture was heated under reflux on the water bath for four hours and then evaporated *in vacuo* at room temperature to half its bulk. Water (30 ml.) was then added and the mixture extracted with ether (4 lots of 5 ml. each). The extract was shaken first with water (5 ml.), then with sodium bicarbonate solution (5%; 5 ml.), and then with water (5 ml.), and dried over anhydrous calcium chloride. After removing the ether by fractional distillation, using a short Vigreux column, the residue was distilled from glass wool under reduced pressure into a trap kept at —30°. A short fore-run (b.p. up to 50°/11 mm.) was discarded and the remaining distillate, nearly all of which boiled at 85-7°/11 mm., was collected as one fraction and used directly in the next stage (yield, 4.74 g.).

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* This paper was written during the tenure of a Squibb Post-doctoral Fellowship in Pharmacology. Present address: School of Chemistry, The University, Leeds 2, England.

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DL-ASPARTIC ACID-α-$C^{14}O_2H$

The crude ethyl malonate (4.74 g.) was converted into ethyl formylaminomalonate in the manner described by Galat (*loc. cit.*). The crude ethyl formylaminomalonate obtained in this way usually contained so much zinc formate that it was not practicable to distill it directly, as suggested by Galat. Therefore, this crude product was shaken with dry ether (30 ml.) and the residual zinc formate filtered off and washed with ether (3 lots of 10 ml.). Evaporation of the ether from this solution and distillation of the residue *in vacuo* gave ethyl formylaminomalonate (3.05 g., b.p. 126°/1 mm.) which was condensed with ethyl chloroacetate and the crude product hydrolyzed to yield DL-aspartic acid-α-$C^{14}O_2H$ (1.22 g.), Galat's directions being followed throughout. (This hydrolysis was conducted in a stream of nitrogen which, after leaving the reflux apparatus, was led into a well-stirred saturated solution of barium hydroxide (800 ml.); in this way most of the $C^{14}O_2$ which was evolved was recovered as barium carbonate-C^{14}.)

OROTIC ACID-$C^{14}O_2H$

The crude aspartic acid described above (1.22 g.) was used without further purification for the synthesis of orotic acid. The method of Nyc and Mitchell (*loc. cit.*) was employed. Two stages of this synthesis deserve comment. (a) When DL-ureidosuccinic acid in amounts less than 1 gm. is evaporated with 20% hydrochloric acid, as described by these authors, the reaction time usually is not long enough to effect complete cyclization and a large part of the starting material is recovered. Best results are obtained by heating this reaction mixture under reflux for 20 minutes and then evaporating it to dryness *in vacuo*. (b) In describing the oxidation of 5-(carboxymethyl)-hydantoin* to 5-(carboxymethylidene)-hydantoin, Nyc and Mitchell refer to an amount of bromine which is only 31% of that theoretically required. A mixture of 5-(carboxymethyl)-hydantoin (0.640 g.), bromine (0.660 g., 1.02 mols), and glacial acetic acid (3.3 ml.) when caused to react as described by these authors gives 5-(carboxymethylidene)-hydantoin in 78% yield.

The orotic acid monohydrate (0.410 g.) thus obtained had a specific activity of 18,000 counts per minute per mg. (c./m./mg.), when counted† as an infinitely thin film of anhydrous material with an end-window counter. Its ultraviolet absorption spectrum in water was identical with that recorded by Nyc and Mitchell (*loc. cit.*). An ascending paper chromatogram made with this product on Whatman No. 1 paper in a *n*-propanol-formic acid-water medium, as described by Leone and Scale,[8] when scanned in ultraviolet light showed only one spot, with R_f 0.45 identical with that shown by an authentic sample of orotic acid run concurrently. Radioactivity measurements showed that there was only one radioactive spot and that this was coincident with the spot visible under ultraviolet light.

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* Referred to earlier as 5-(acetic acid)-hydantoin (*cf.* Lieberman, I. and Kornberg, A.: *J. Biol. Chem.*, 1954, *207*, 911.

† I am indebted to Miss Beatrice A. Karger for this measurement.

DECARBOXYLATION OF OROTIC ACID-$C^{14}O_2H$

The radioactive orotic acid described above was diluted with inactive orotic acid and dried at 130°/1 mm. to constant weight. A portion of the product was oxidized by the Van Slyke-Folch method and the resulting carbon dioxide counted as barium carbonate using an end-window counter. The specific activity was 2790 c./m./mg. of anhydrous orotic acid.

Weighed portions of this diluted acid were heated to 345° for periods of five minutes each in a potassium nitrate-sodium nitrite bath, in a current of dry, oxygen-free nitrogen. The carbon dioxide evolved was determined by absorption in a tared "bubbler" containing sodium hydroxide (2 *N*); found: 1.15±0.05 mol. of carbon dioxide per mol. of orotic acid. At the conclusion of each experiment the absorbed carbon dioxide was precipitated in the usual way as barium carbonate; this was plated out and counted under conditions identical with those of the oxidation experiment. The mean of several such determinations gave a specific activity of 2545 c./m./mg. of anhydrous orotic acid, i.e., under these conditions, 91% of the radioactivity of the orotic acid was lost as carbon dioxide.*

The dark residue left in the reaction tube at the completion of the pyrolysis was made up to a given volume with dilute aqueous ammonium hydroxide and an aliquot of this solution was chromatographed on Whatman No. 1 paper using the n-butanol-water medium of Vischer and Chargaff.[6] When screened in ultraviolet light the paper showed some light-absorbing material which had remained stationary, and one spot with R_f 0.37, the same as that of an authentic sample of uracil run concurrently. The spot was eluted with dilute aqueous ammonium hydroxide. A portion of this solution when examined spectrophotometrically showed absorption spectra at pH 1 and pH 13 identical with those described for uracil[1] and showed that 0.88 ± 0.05 mol. of uracil was formed per mol. of orotic acid pyrolyzed. Another portion of this solution, when plated out as an infinitely thin film (0.4 mg. uracil per planchet of area 3 $cm.^2$) and counted under the same conditions as are described above, was not detectably radioactive.

SUMMARY

The preparation of orotic acid-$C^{14}O_2H$ is described. $BaC^{14}O_3$ (150 mg., 5 mc.) was converted, via sodium cyanide, into malonic acid and the corresponding ethyl ester, and the latter was converted into ethylformylaminomalonate. After condensation with ethylchloroacetate the product was hydrolyzed to give DL-aspartic acid-α-$C^{14}O_2H$ and this was converted into orotic acid monohydrate via DL-ureidosuccinic acid, 5-(carboxymethyl)-hydantoin, and 5-(carboxymethylidene)-hydantoin. The orotic acid-$C^{14}O_2H$ monohydrate (410 mg.) had a specific activity of 18,000 counts per milligram when counted with an end-window counter and appeared to contain the radioactivity exclusively in the carboxyl group.

* The error of this result is estimated as ±5%.

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BOOK REVIEWS

HISTOLOGY. Roy O. Greep, Ed. New York, Blakiston, 1954. 953 pp., 648 figs., $15.00.

This massive textbook of histology marks a new departure from the usual text. It began as a sixth edition of the text by Bremer and Weatherford and ended as a completely new book, both as to text and as to most of the illustrations. In addition, the chapters have been written by thirteen different contributors, all of them associated in one way or another with the Department of Anatomy at Harvard Medical School. The decision to rewrite the old text entirely as well as to enlist the aid of experts in the various branches of histology has proved to be a wise one, for this is certainly one of the best of the newer texts on the subject.

This book is written in an easy, simple manner, progressing from basic considerations of cytological structure through the tissues to the more complex organs and organ systems. Each chapter builds upon the information and concepts presented in the previous one in a manner which reveals the excellent judgment of the editor. The tone of the book is set by the early chapters which emphasize recent developments in histochemistry and electron microscopy. New information is integrated with classic concepts smoothly and clearly throughout the book. Correlation of structure with function is repeatedly emphasized. A welcome addition to the subjects included in histology is the chapter on placentation by G. B. Wislocki. Other embryological considerations are integrated into the chapters dealing with specific organs and tissues.

The task of welding all of these contributions into a coherent text has resulted in some needless repetition which may be removed in succeeding editions. There are also some distracting errors which should be readily recognized and corrected even by the student-reader. Others may be rather confusing, such as the statement on page 164 that the ciliary muscle of the eye is striated, but not under voluntary control. Some errors are merely amusing, such as the statement on page 209 that Nissl was a *Dutch* histologist, which will be surprising news to neurologists who are aware of Nissl's strong German patriotism. Throughout the text are small errors in English, like the use of *media* as a singular noun (p. 914). But these are relatively small slips in a text of such general excellence.

It is deplorable that a text for first-year medical students should be priced at fifteen dollars. Undoubtedly, the large number of excellent illustrations contributes to the high cost, but the publishers should be able to find ways to reduce the price at least to that of the nearest competitor. The publisher has recognized his responsibility in this respect by offering to sell this text together with two others used by first-year medical students at a reduced price if bought together. Unfortunately, such tactics do not give this fledgling a fair opportunity to demonstrate its appeal.

SANFORD L. PALAY

PERIPHERAL CIRCULATION IN MAN. Ciba Foundation Symposium. G. E. W. Wolstenholme, Jessie S. Freeman, and Joan Etherington, Eds. Boston, Little, Brown and Co., 1954. xii, 219 pp. $6.00.

The Ciba Foundation's symposium on "Peripheral Circulation in Man," was held in May, 1953. Under this easy title are gathered together eighteen

short papers on the subject with full discussion. These include: "A critical survey of methods available for the measurement of human peripheral blood flow," by A. C. Burton; "The venous occlusive technique for measurement of finger blood flow," by G. E. Burch, Jr.; "The electrical strain gauge method for measurement of peripheral circulation in man," by R. J. Whitney; "The transparent chamber technique for observation of the peripheral circulation, as studied in mice," by G. H. Algire; "Differential secretion of noradrenaline and adrenaline from the suprarenal gland," by U. S. von Euler; "The effect of adrenaline and noradrenaline on the blood flow through human skeletal muscle," by R. F. Whelan; "Changes in peripheral circulation with exposure to cold," by L. D. Carlson; "The response to cold in the range 0-10° C.," by A. D. M. Greenfield; "Factors concerned in the regulation of skin blood flow," by D. McK. Kerslake and K. E. Cooper; "Blood flow response to temperature and other factors," by H. Barcroft and A. C. Dornhorst; "Observations on the neurohistology of cutaneous blood vessels," by G. Weddell and W. Pallie; "Reflex control of the cutaneous circulation," by K. E. Cooper and D. McK. Kerslake; "The problem of vasomotor denervation," by A. B. Hertzman; "Vascular reactivity following sympathectomy," by R. T. Grant; "Some anatomical observations on peripheral ischaemia in man," by J. H. Dible; "The mechanism of the Raynaud's phenomenon in patients with high serum titres of cold agglutinins," by J. T. Shepherd; "Visceral activity and peripheral circulation in the spinal man," by L. Guttman; "Some aspects of functional disorders of the circulation," by P. Martin.

The complexity of problems relating to the peripheral circulation is indicated by the above list, and this impression is forcibly brought home as one reads the ensuing pages. Any simple, mechanical concept of the circulation is wholly inadequate for the understanding of its function in health or disease. The peripheral segment, far from approaching the conventional pump-and-pipe model, is in fact more like a slough, surrounded by a bog, encompassed by a swamp. Its volume and flow are influenced by vagaries of the structure and position of the anatomical landscape; they vary with physical and chemical changes in the local and distant body weather and once changed may develop new patterns that persist. Nothing is rigid, nothing is geometrical, little is absolute.

How to make meaningful measurements in a situation of this sort is the problem that engages the attention of the investigators in this symposium. The challenge is of course basic in any sort of experimental or clinical science. Repeatedly the participants wish to know what quantity or quality of tissue is measured by a particular method, its "intrinsic variable," what the relation of this "intrinsic variable" is to the "target variable," the quantity or quality that is actually sought in the experiment, and finally what is the "reactive error" associated with the changed state of the organism induced by the method. Viewed in the light of such rigid, self-imposed critique, the physiological observations presented are probably valid as indicating at least ranges and trends in variables of the peripheral circulation. They will become useful for further experimentation and for eventual clinical application.

The book, as a whole, by reason of the simplicity and brevity of the presentations, and the wise selection of references, will be of interest to the beginner in the field of peripheral circulation as well as to the expert, who may better appreciate the advanced discussions. For anyone, it is a lesson in scientific approach.

LEVIN WATERS

ENDEMIC GOITER. The Adaptation of Man to Iodine Deficiency. By John B. Stanbury, Gordon L. Brownell, Douglas S. Riggs, Hector Perinetti, Juan Itoiz, and Enrique B. Del Castillo. Cambridge, Massachusetts, Harvard University Press, 1954. 209 pp. $4.00.

This monograph reports the results obtained by the Andean Goiter Expedition, a joint project undertaken by Harvard Medical School, the University of Cuyo, and the Central Hospital of Mendoza, Argentina. The object of the venture was to use modern analytical and radioactive tracer techniques for studying the metabolism of iodine and the alterations of thyroid function in human beings living in an area of iodine deficiency. The opportunity for such studies is limited, both because there are only a few places where iodine deficiency is well established and because the widespread use of prophyllactic iodination of salt or other common articles of diet has cured the deficiency in most of the accessible areas where it was known to exist. The sale of iodized salt has been made mandatory in Mendoza, the subject of the present observations, since the expedition completed its work. The present work therefore probably represents one of the last opportunities to study endemic iodine deficiency. Knowing this, the authors have brought their full experimental armamentarium to bear on the study of the effects of iodine deprivation on thyroid function, and also the effects of various pharmacological and dietary influences on the altered thyroid metabolism. The methods used for experimental and mathematical analysis are clearly presented, and the observations are given in great detail. The book is well-organized, with separate sections for methodology, observations, and interpretation. At the end of each chapter there is a brief bibliography and a summary. Each chapter therefore can be read as a separate paper. The sections concerned with developing a mathematical model for thyroid function are set forth in simple style, and the limitations of the model are described explicitly. The last chapter summarizes the entire work.

Although the study concerns a very special and unusual clinical situation, the information obtained is of basic importance to the understanding of normal thyroid physiology. The clinician will be particularly interested in the relative efficacy of iodide and dessicated thyroid in suppressing endogenous thyroid activity, in the observations on the mechanism of action of methimazol, in the description of the development of hyperthyroidism in a single case after the administration of large amounts of iodide to a previously deficient patient (iodbasedow), and in the ability of the iodine-deficient thyroid to maintain normal levels of hormonal activity. The book will be useful to anyone interested in thyroid function.

PHILIP K. BONDY

THE MEANING OF SOCIAL MEDICINE. By Iago Galdston. Commonweath Fund. Cambridge, Harvard University Press, 1954. 137 pp. $2.75.

The author is impatient with a Medicine that cures disease but does not bestow health, and urges a reorientation to concern with life and its fulfillment rather than with death and disease. The current dilemma is blamed upon a faulty philosophy of medicine traced to the influence of Pasteur and von Behring, who led into the search for single causes of disease and disregarded broader concepts of etiology that considered individual variations and environmental factors affecting the health of the individual. The trend led to "curative" medicine that does not promote health and has left us with an ever-increasing burden of cured people who remain unhappy, unproductive, and physically or emotionally infirm. Dr. Galdston is highly critical of contemporary medical education which continues to teach an unsuitable approach and prepares the physician of tomorrow to practise a medicine that was inadequate yesterday.

The criticisms of medicine are often justified and are very well stated, but the historical analysis of the situation seems biased and misleading. However, the major fault of the book is that its purpose, as stated in the title, remains unfulfilled. Many usages of the term "Social Medicine" are offered in the opening chapter, but the divergent definitions are never resolved. The author's closest approach is found in his appeal for comprehensive, patient-oriented medicine, but does not include the field which many workers consider to be the particular province of "Social Medicine" —the statistical correlations between social factors and the health of populations and the analysis of such data.

The book contains arguments that seem unnecessary to state the case for social medicine and some of them seem prejudiced to the extent of being antagonizing. The emphasis upon the bacteriologists as the spiritual fathers of modern medicine shows a disregard for Claude Bernard and his followers, whose primary concern with the consideration of the homeostasis of the organism has thoroughly permeated medical school teaching. Strangely, the social change that has made the need for a new orientation to medical practice critical, and the rôle that "curative medicine" has played in producing this change in social conditions is not considered adequately. The critique of American medical education, while often germaine, displays an unfamiliarity with current trends. It is a gross overstatement to write, "the goals and objectives of medical education have not changed since the time of Sydenham. They remain: to teach the novitiate to recognize the presence of disease, to be able to diagnose it correctly, i.e., to fit it into some nosological category, and to treat the disease (or the diseased person) so that disease is eliminated or its malign effects are countered or reduced." Basic alterations in education are necessary to prepare the student with broader concepts and abilities. It does not help to misrepresent the situation that needs to be changed. The leaders of American medical education include many persons who are aware of the task before them. Dr. Galdston has only stated the need. He has not offered any practical guides.

THEODORE LIDZ

FEELINGS AND EMOTIONS. By Lawrence K. Frank. 38 pp.; INTELLIGENCE—STATISTICAL CONCEPTS OF ITS NATURE. By L. J. Bischof. 33 pp.; PERCEPTION—A TRANSACTIONAL APPROACH. 33 pp. Garden City, New York, Doubleday and Co., 1954. 85 cents each.

These booklets, in a series called the Doubleday Papers in Psychology, are evidently intended for supplementary reading in undergraduate courses in psychology and as separate treatments of particular topics of interest to the general reader. The basic idea seems to me a good one, for an instructor, or a layman seeking enlightenment on a particular topic, could from such a series secure what he wants without having to use, purchase, or plow through other materials not of interest at the moment. The execution of this good basic idea is of course no easier than the writing of the perfect textbook. Thus it is that the three papers under review do not seem at all equal in quality or in likelihood of contributing to the purpose of the series.

The paper by Frank treats of emotions first as a mammalian function, and as an aspect of homeostasis. The bulk of the paper, however, concerns emotional development, treated with enough generality that many would call this a discussion of personality development. Special attention is given to early processes of emotional response, to the socialization of emotion, to the rôle of language in emotional development, and to the major problems in adolescence. Much clear sense is made of the origins of emotional conflict, repression, and society's influence upon the learning of emotions. Emotional maturity and emotional integrity are given sensible and realistic interpretation. The chief intellectual influence is perhaps psychoanalysis, but elements of Lewin's psychology, the biology of growth, and other ideas are also apparent and well integrated into the author's view. His language is well selected, the technicalities of psychoanalytic terminology, for example, being avoided without losing the rich meanings of the concepts. To me, this paper is a fine example of how different disciplines and concepts can be brought to bear upon a selected topic so that an elementary student or a parent can read with little aid, but with substance enough that the experienced scholar can profit.

Bischof's paper consists of a brief introduction to the history of intelligence testing and the I.Q., to selected *quantitative* theories of intelligence which are grouped as defining intelligence as problem solving, and then some elaboration of the Thurstone primary mental abilities approach, together with presentation of a sample test and a brief cataloging of available tests. The paper is rather clearly written. It utilizes a pictorial rather than a statistical presentation, because of the complexity of the statistical ideas which are necessary to deal with the quantitative theories at their own level. My objections to the paper center about the fact that the audience which it should serve is not clear: if this is a beginning-course audience, then the variety of theories, several outmoded, is not necessary, and more concentration on the relation of intelligence to other variables is desirable; if it is an advanced-student audience, the statistical nature of the theories really ought to be met directly, not avoided by pictorial means; if it is a non-psychologist audience, there is not enough concern for the uses and

misuses of intelligence tests, in addition to the objection already registered for the beginning-course audience. (I also feel that the pictorial method and the text both fail to communicate the views of Thomson and Thorndike adequately.)

The paper by Ittelson and Cantril contains a general discussion of the nature of perception, methods for studying it, its rôle in living, and its characteristics in operation. It is well written and clearly organized, and thus makes available a brief treatment for those who want to know what the Princeton group is up to. Their "transactional" view is utilized and the main purpose of the paper is to explain it. The authors say this view has its roots in the writings of Dewey and Bentley; it stresses the interdependence of all aspects of a perceptual situation, including the fact that perception is an active process in some perceiver. Perception is said always to be unique (skirting dangerously close to the position which would remove perception from the realm of scientific study by denying the possibility of deriving laws, i.e., statements of some degree of generality about it), and also to exhibit the feature of externalizing, so that the world we experience is the product of perception, not the cause of it. My reactions to this as a view are colored by my reading of other publications by the Princeton scholars and their forerunner, Ames. I find them difficult to read because of the use of many common words with local definitions which take time to absorb: significances, except-fors, hitch, form and flow, are but illustrations of the many words which have to be partially re-defined or delimited before one can read this writing. More important, I fail thus far to find much that is new or different in the transactional view. It contains elements of Gestalt reasoning and of Allport's views, and is markedly influenced by Ames and his demonstrations, but it does not in my opinion deal adequately with the theory of perceptual development (which it should by its nature), with how perception relates to judgment, or with the rôle of language in perception.

I cannot resist a final remark: the editor's introductions to the papers are uneven in character; furthermore, that in the Frank paper is over-inclined to exalt the author, while that in the Ittelson-Cantril paper exalts the view. There is no need for this.

CLAUDE C. BUXTON

THE METABOLISM OF ALGAE. By G. E. Fogg. New York, John Wiley and Sons, 1953. 149 pp. $2.00.

This excellent monograph, citing over 300 references to algological literature, surveys the field of algal metabolism—the chemical composition of and transformations brought about by algae—from the viewpoint of comparative biochemistry. It is not a general physiology; there are, for example, no discussions of sexual substances or processes, phototaxis, nor dormancy.

Up to the present there have been but a half dozen-odd books or major articles dealing with algal physiology. The publication of this book indicates that there is now a sufficient body of data on algal metabolism to make

necessary the same systematic treatment that has been given the metabolism of bacteria, fungi, and the higher plants.

The book is divided into eight chapters including an introduction and summary. There are sections on respiration, photosynthesis, the occurrence and distribution of pigments, heterotrophic nutrition, requirements for amino acids, vitamins, and other growth factors, and nitrogen metabolism, including nitrogen fixation. The formation and nature of algal carbohydrates, proteins, lipids, and sterols are reviewed, and the differences are considered in relation to phylogeny. One chapter is concerned with the effect of growth conditions in culture or in nature upon the quantity and composition of these end products.

This book will be of value to the student of algal ecology and physiology as well as to the comparative biochemist or physiologist.

R. R. L. G.

CHEMOTHERAPY OF INFECTIONS. By H. O. J. Collier. New York, John Wiley and Sons, 1954. xvi, 248 pp. 50 figures. $4.00.

The saying that good things come in small packages might well apply to this modestly dimensioned British contribution to the chemotherapeutic field. With a proper scorn for superfluity and based on the premise that "the action of chemical substances upon microbes and man and, in turn, the reactions of host and parasite to chemicals form a triangular relationship," Dr. Collier has in the space of 230 pages dealt admirably with his subject. The foreword by Sir Alexander Fleming initiates what proves to be the mood of the entire work, i.e., that while an adequate discussion of chemotherapy necessarily includes elucidation of basic pripciples and incorporation of all latest pertinent information, yet, to be complete, historical and developmental aspects must not be overlooked.

The book is divided roughly into three areas. The first deals chiefly with general considerations such as "The Problem of Getting the Chemical to the Microbe" and "The Development of Resistance"; the second discusses the various chemical agents individually, while the third takes up the problem of the chemotherapy of specific diseases.

Its diminutive size notwithstanding, this book should be a valuable addition to any medical library.

J. C. C.

THE ATOM STORY, BEING THE STORY OF THE ATOM AND THE HUMAN RACE. By J. G. Feinberg. Foreword by F. Soddy. New York, Philosophical Library, 1953. vi + 243 pp. $4.75.

The Atom Story, to quote from its jacket, "is the first complete and balanced book on the Atom in the language of the layman." Balance is given to the book by the fact that it is not concerned solely with the atomic bomb but with the historical evolution of atomic theory from Democritus to the present, and indeed, with the future of atomic power in peace and war. The book, therefore, really has three sections: the history of atomic theory up

to the twentieth century; the theoretical and technological background of the bomb; the prospects for atomic energy in its peaceful guise and methods of survival in an atomic war (this being Appendix A—a reprint of the Office of Civil Defense's pamphlet *Survival Under Atomic Attack*). Since these sections are not uniform in quality, they had best be passed under review individually.

The first 90 pages or so are devoted to the fortunes of the atomic theory through the centuries. But since the real object of the book is to explain the atom today, the emphasis in this first section falls on those people throughout history who were "right" (i.e., believed in atoms) regardless of their reasons for being "right." This kind of approach inevitably leads to distortions of history and science for it ignores the fact that only in relatively modern times was the empirical evidence forthcoming which gave an atomic theory a larger claim to physical truth than the speculations (all equally logical) of the philosophers of nature from Democritus on. And all this is told in a breathless style which borders on self-parody. The following selection, dealing with Dalton's rejection of Gay-Lussac's suggestions on combining volumes of gases may serve as an illustration (p. 34).

> For the want of a word a scientific kingdom was lost by Dalton! That word was, yes, *molecule*. Dalton was dealing with *molecules*, but knew it not and called them *atoms*. And in this case a rose by another name smelled not so sweet in Dalton's nostrils, as he sniffed speculatively at the proposition of Gay-Lussac.

This reviewer would advise all readers simply to skip the first 90 pages.

Fortunately, the remaining two-thirds of *The Atom Story* is quite different from the "historical" part. Here Mr. Feinberg, a science reporter, is on firmer ground and shows it. His description of the development of atomic theory in the twentieth century is admirable, concise and dramatic. He has quoted extensively (one suspects from personal interviews) from great living scientists and has managed to make this part of his story what the book jacket claims: "a journey of adventure and discovery as intriguing as a well-told cloak-and-dagger yarn."

The last section on atomic power in peace and war is particularly good. Mr. Feinberg deals well and justly with both the optimists who see the atomic pile as the panacea for all of the world's ills, and the pessimists who predict the end of the world by the end of the year.

To summarize, *The Atomic Story* is a good *oeuvre de vulgarisation* if one skips the first ninety pages. It should give the intelligent layman an idea of the complex field of theoretical and applied atomics and Mr. Feinberg deserves credit for attempting to sum up, in such a slim volume, the entire "story of the Atom and the Human Race."

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